BONE MARROW BIOPSY IN BREAST CANCER

A clinical and morphological study

by

Karl Landys

Göteborg 1980

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BONE MARROW BIOPSY IN BREAST CANCER

A clinical and morphological study
based on a survey of 622 cases

AKADEMISK AVHANDLING

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KARL LANDYS

Göteborg 1980

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LANDYS, K., Department of Radiotherapy, University of Göteborg, S-413 45 Göteborg, Sweden

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II. Landys, K.: A new Trephine for Closed Bone Marrow Biopsy. Acta Haematol. Basel, Vol 64, Nr 4, in press 1980.

III. Ridell, B. and Landys, K.: Incidence and histopathology of metastases of mammary carcinoma in biopsies from the posterior iliac crest. Cancer 44: 1782–1788, 1979.

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Key words: breast cancer – Bone marrow biopsy – Clinical and morphological correlation – Bone scintigraphy – Prognosis

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From the Department of Radiotherapy, Sahlgrenska sjukhuset,
University of Göteborg, Sweden

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1 INTRODUCTION

1:1 Historical review

Microscopic examination of the bone marrow constitutes an essential measure in the investigation of many malignancies, such as leukaemias, lymphomas and small-cell anaplastic carcinoma of the lung.

The routine method of investigation of the bone marrow is by aspiration cytology. The first study of the microscopic structure and function of the bone marrow was published in 1886 by Bizzozero. The use of the technique for clinical purposes was described by Wolf (49) in 1903 and Pianese (38) in 1905. Seyfarth (42) in 1923 and Arinkin (1) in 1929 published papers on sternal puncture and this technique rapidly became a routine procedure in the investigation of blood diseases and made important contributions to the development of haematology. Lethal complications of sternal puncture in the early 1940s led to technical improvements to the instrument and to a search for less hazardous puncture sites.

In 1945 van den Berghe and Blitstein (3) began puncturing the anterior iliac crest. In a comparative study of sternal puncture and puncture of the posterior iliac crest, Rubinstein (41) showed in 1948 that the latter technique was associated with fewer complications. This method has therefore become increasingly common and the posterior iliac crest is now considered to be one of the safest puncture sites. No lethal complications have been reported up to now. Moreover, this site provides the possibility of obtaining sufficient marrow for histopathological investigation.

Several subsequent studies have shown that histopathological investigation of drilled marrow specimens is superior to aspiration biopsy for diagnosis of metastases of solid tumors (8, 11, 21). The microscopic verification of bone marrow metastases may be considered as a valuable complementary method in the clinical investigation (29, 45). Among the many workers contributing to the technical and diagnostic development of bone marrow biopsy Dameshek (13), Silverman (43), Burkhardt (7), Radner (40), Gidlund, Landys & Stenram (30), Bordier (5), Stavem (46), Miller and Dennis (35), Jamshidi, Windchitl & Swaim (26), and Fornasier & Vilaghy (19) deserve special mention.

1:2 Bone marrow biopsy in patients with breast cancer

Relatively little is known concerning the value of bone marrow biopsy as a diagnostic procedure in patients with breast cancer, one of the commonest tumor diseases (24, 25). Its incidence increases exponentially with age from the third to the fifth decade of life (17). About one half of all women who develop breast cancer die due to dissemination of the disease. There is growing evidence that breast cancer is a systemic disease more often than can be detected by routine staging procedure (18). It is also known that the efficcacy and the type of treatment vary with the extent of the tumor. Early detection of the metastatic tumor is therefore extremely important.

1:3 Hypothesis for probability of early diagnosis of various types of osseous metastases

Osseous spread of solid tumors can vary topographically, substantially and morphologically. It is a dynamic process and is too complicated to be explored by means of routine methods of investigation. Fig. 1 demonstrates various hypothetical types of development of osseous metastases and the possibilities of verifying them by biopsy from the iliac crest:

Type A represents a hypothetical origin of the spread which cannot be revealed by either radiography, bone scintigraphy or biopsy.

Type B represents a solitary skeletal metastasis revealed by bone scintigraphy, radiography or both.

A and B represent a minimal tumor mass which can possibly be cured. When the metastases grow, different degrees of pelvic invasion are possible, as suggested in

types C, D, E, F, G and H, all of which worsen the prognosis. Type A tumors can progress to types E, F, G and H or to types B, C, G, H, and C–D. Type B tumors can develop type C, D, G and H metastases. All depicted situations except A and B seem to have a poor prognosis, with increasing involvement of bone marrow. This may be explained by the "host resistance decompensation" followed by accelerated growth of the metastases, vital organ involvement and death, as suggested by Pawlias and his collegues as early as 1958 (37). Types B, C, D, G and H are usually diagnosed by bone scintigraphy, radiography or both. Types A, E and F may remain clinically undetected for long periods. Some of the type E and F metastases can probably only be revealed by bone marrow biopsy. Metastases of type G and H may be easier to recognize by radiography, bone scintigraphy and also by bone marrow biopsy, because of the larger tumor mass than types A, B, C, D and E.

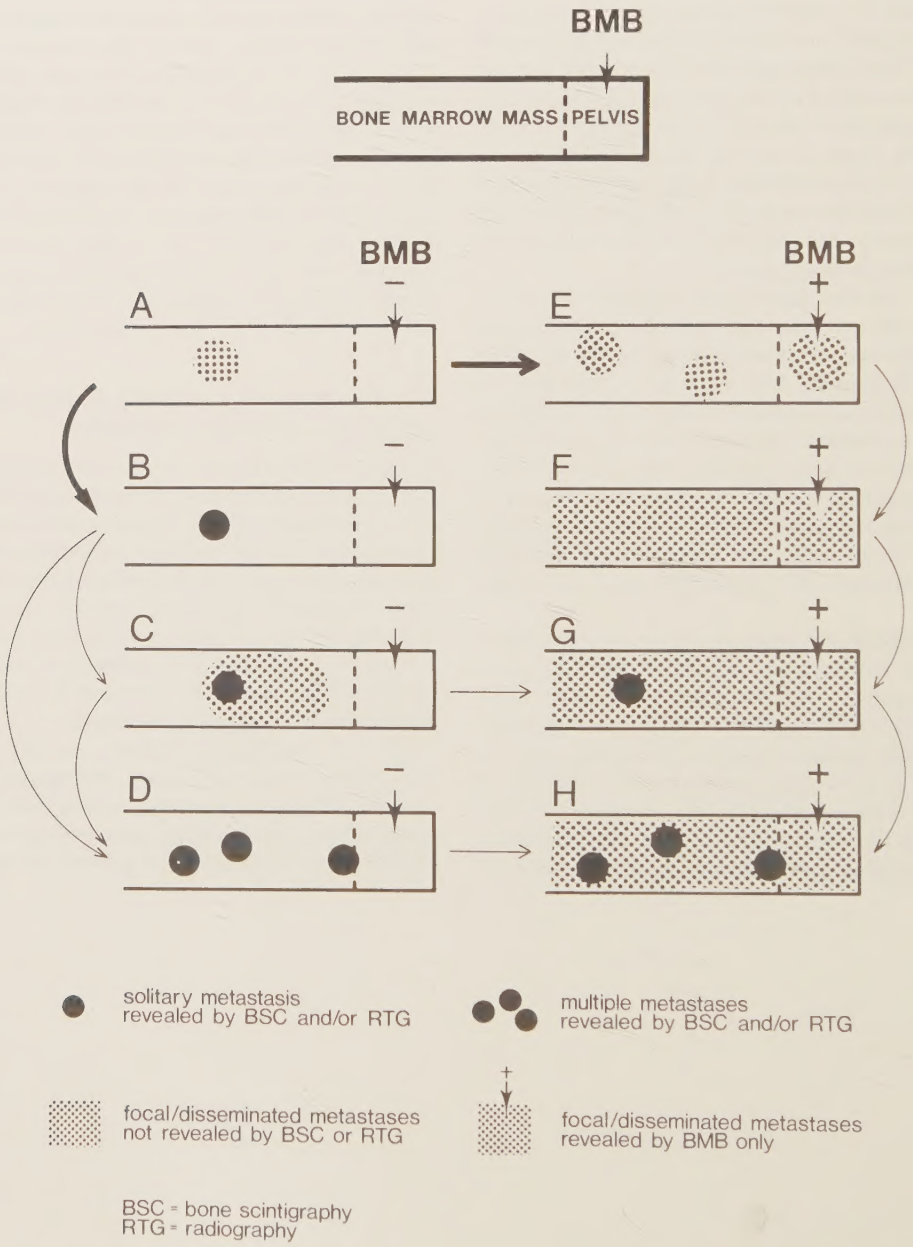

Fig. 1. Conditions for blind bone marrow biopsy (BMB) in various types of osseous metastases

2 AIM OF PRESENT STUDY

The aim of this study in patients with breast cancer was to determine:

- Whether bone marrow biopsy can contribute to the clinical examination;
- The incidence and histopathology of bone marrow metastases in the posterior iliac crest;
- Whether bone marrow biopsy has prognostic value, and
- whether the morphology of metastatic tumors in bone marrow is related to the survival of patients with advanced disease.

3 PATIENTS AND METHODS

Of 1950 patients with primary and 503 patients with metastatic breast carcinoma treated at the Department of Radiotherapy, Göteborg between 1975 and 1979, 622 patients were examined by bone marrow biopsy (BMB) and 332 of these also by bone scintigraphy (BSC) as a part of the routine diagnostic work-up at the time of initial diagnosis or during follow-up 6 months – 6 years after surgery, see Fig. 2. The mean age of the BMB patients was 59.1 years (range 27–85) and the mean age of the BSC patients was 58.5 years (range 27–83) see Fig. 3. Only outpatients were included in this study. The malignancy of all primary tumors was verified by microscopic examination.

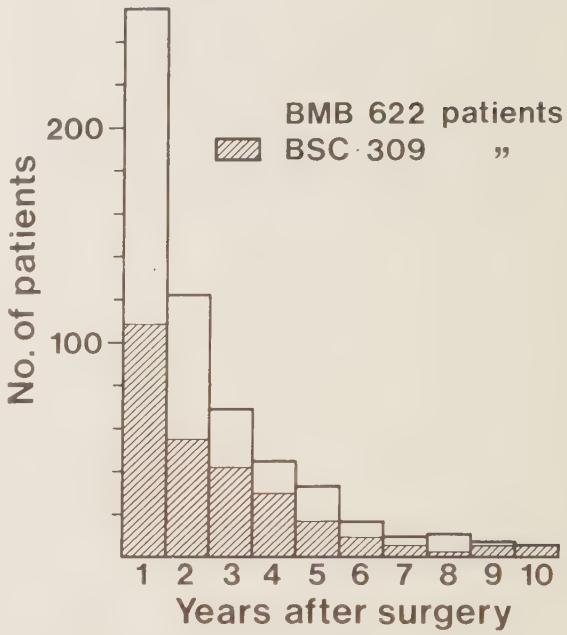

Fig. 2. Bone marrow biopsy (BMB) and bone scintigraphy (BSC) in relation to the time of surgery

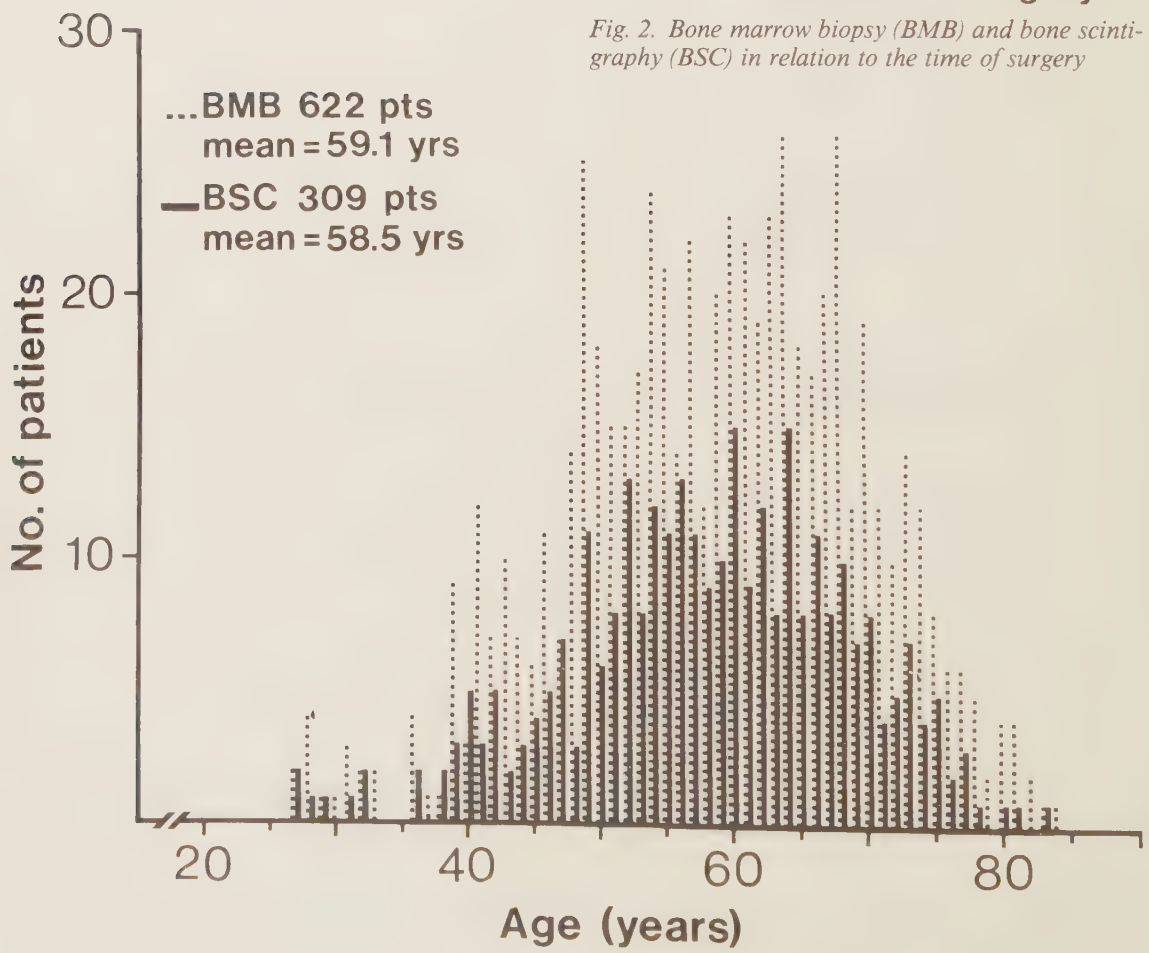

Fig. 3. Distribution of patients by age

3:1 Clinical diagnosis and staging procedure

All patients were clinically staged according to the UICC TNM – classification of 1972 (48).

The diagnostic work-up comprised a case history, physical examination and investigation of blood haemoglobin, white blood cells, platelets, peripheral blood smears, serum electrolytes, urinalysis and liver function tests.

3:2 Radiography

The routine radiographic examination included the chest, thoracic and lumbar spine and pelvis.

3:3 Patient distribution

The patients were divided into two groups according to TNM stage, see Table 1.

The first group consisted of 415 patients with operable tumors, 60 stage I, 247 stage II and 108 stage III.

The second group comprised 207 patients with distant metastases, stage IV carcinoma.

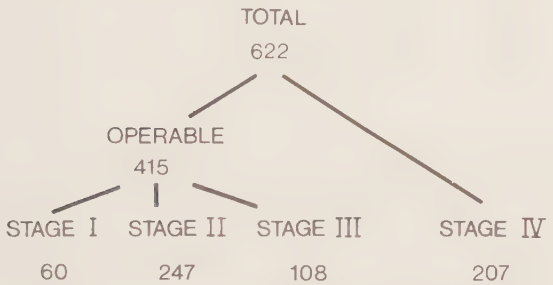

Table 1. Distribution of patients by operability and stage

3:4 Operable tumors stage I–III

The patients in this group were further subdivided according to the following clinical and pathological criteria:

1) Primary tumor location;
2) Primary tumor size and axillary node histopathology;
3) Hormonal status;
4) Time of BMB

3:4.1 Primary tumor location

The primary tumor location in patients with operable breast cancer is shown in Table 2.

3:4.2 Primary tumor size and axillary node histopathology

The clinical stage has been studied in relation to the size of primary tumors, obtained by measurement of the greatest diameter in the operation specimens (Table 3), and the incidence of histopathologically diagnosed axillary metastases (Table 4).

ZONE	No. OF PATIENTS
CENTRAL	49
UPPER + LOWER INNER	114
UPPER + LOWER OUTER	252
TOTAL	415

Table 2. Primary tumor location in patients with stage I–III operable breast cancer

CLINICAL STAGE	MAXIMAL DIAMETER OF PRIMARY TUMOR No. OF PTS		
	< 2 cm	2 – 5 cm	5 – 7 cm
I	59	1	0
II	21	225	1
III	6	27	75
TOTAL	86	253	76

Table 3. Clinical stage in relation to the tumor size stage I–III

CLINICAL STAGE	No OF PTS	AXILLARY NODE METASTASES		AXILLA NOT EXPLORED
		YES	NO	
I	60	0	52	8
II	247	72	117	58
III	108	74	30	4
TOTAL	415	146	199	70

Table 4. Clinical stage in relation to the incidence of axillary node histopathology

3:4.3 Hormonal status

One hundred and fifty-four women were premenopausal and 261 were postmenopausal.

A woman was considered to be postmenopausal when two years had elapsed since her last menstrual period.

3:4.4 Time of bone marrow biopsy

BMB was performed in 103 patients in stage I–III at the time of intial diagnosis and in 312 patients during follow-up 6 months – 6 years after surgery and/or radiotherapy, Table 5.

STAGE	No OF PATIENTS	
	AT THE TIME OF INITIAL DIAGNOSIS	IN FOLLOW-UP $\frac{1}{2}$ – 6 YEARS AFTER OPERATION
I	18	42
II	65	182
III	20	88
TOTAL	103	312

Table 5. Clinical stage in relation to the time of bone marrow biopsy, operable tumors

3:4.5 Treatment of patients with stage I–III

Two hundred and seventy-nine patients were subjected to modified radical mastectomy, 66 radical mastectomy ad modum HALSTED and 70 simple mastectomy.

One hundred and seventy-two patients were irradiated postoperatively. Oophorectomy, surgical or radiological, was offered to all premenopausal patients with a high risk of metastases (advanced local disease, extensive axillary node involvement).

3:5 Stage IV tumors

Of the 207 patients in stage IV, 96 had radiographically revealed osseous metastases only, 89 non-osseous metastases and 22 had mixed osseous and non-osseous metastases.

In none of the 96 patients with *osseous metastases* was there a clinically evident spread to other sites at the time of BMB and BSC. The location of metastases within the skeleton is shown in Table 6. Seventy-eight of these patients were also examined by BSC.

The group of 89 patients with *non-osseous metastases* consists of 44 patients with microscopically verified cutaneous and/or subcutaneous metastases, 30 patients with radiographically revealed involvement of the lungs, 14 patients with metastases in the pleura and 1 patient with a metastasis in the pericardium. The pleural and pericardial metastases were cytologically verified. In 55 of these patients BSC was also carried out.

SKELETAL AREA	SOLITARY LESION	MULTIPLE LESIONS MAINLY IN
LUMBAR SPINE	23	27
THORACIC SPINE	13	4
RIBS	8	3
PELVIS	4	0
PROXIMAL FEMUR	5	1
CERVICAL SPINE	4	0
HUMERUS	4	0
TOTAL	61	35

Table 6. Location of osseous metastases revealed by radiographic examination

Twenty-two patients had *mixed metastases.* Ten of them had metastases in the lungs and 12 in the pleura. All of them had radiographically verified metastases in the ribs or spine, but not in the pelvis. In 17 of these patients BSC was also performed.

BMB was not performed in patients with liver, peritoneal or brain metastases.

3:5.1 Treatment of patients with stage IV tumors

The patients with clinical stage IV disease, positive BSC and positive BMB received combined chemotheraphy ad modum COOPER, modified as follows: Oncovin 1 mg + Methotrexate 25 mg + 5-Fluorouracil 500 mg + Cyclophosphamide 500 mg i.v. once a week. Thirty-three patients also received Primobolan Depot^{R} (Methenolone-enanthate, Schering AB) 100 mg i.m. once a week. Some patients also received palliative radiotherapy.

3:6 Follow-up

All patients were followed up every other month with a physical examination, haemoglobin, WBC, platelets counts, control of serum electrolytes and liver function tests. Radiographic examination of the lungs and skeleton, bone and liver scintigraphy or other diagnostic procedures were performed when clinically indicated. All patients have been followed up for at least 40 months.

3:7 Bone scintigraphy

BSC was performed at the Division of Nuclear Medicine, Sahlgrenska sjukhuset, with a scintillation camera Pho/Gamma III HP (Searle) or Maxi camera II (General Electric), using a low energy, high resolution, parallel-hole collimator. The first 65 patients were examined with ^{99m}Tc pyrophosphate or polyphosphate and the remaining 244 patients with ^{99m}Tc stannous methylene diphosphonate (New England Nuclear). The routine BSC included posterior views of

the skull, cervical, thoracic and lumbar spine, chest and pelvis, the proximal portions of the femoral bones and in some cases frontal views of the chest. The BSC was classified as negative, doubtful or positive, using the following criteria:

Negative = Homogeneous and symmetrical distribution of the radionuclide within the skeleton.

Doubtful = Slightly increaseed activity of radionuclide in one or multiple areas or markedly increased activity in one area only.

Positive = Markedly increased activity of radionuclide in at least two foci.

3:8 Bone marrow biopsy and histotechnique

BMB were taken from the left posterior iliac crest with Gidlund's trephine (30) or a new trephine, called the LM-trephine (Paper II), usually with the patient sitting leaning forward with her arms on the table.

Under local anesthesia using 8–10 ml of 2 % Xylocaine^{R} (ASTRA), a small skin incision was made over the posterior iliac crest about 5 cm from the midline and the bone was trephined at the level of the first sacral vertebra. The incision was closed with a simple skin suture. Every patient was examined ten days after biopsy.

The bone marrow plugs were fixed immediately after removal in 6% formaldehyde solution for 6–24 hours. The decalcification was performed using Parengy's fluid (28) or New Decalc (Betlehem Trading, USA).

The specimens were than treated according to routine histopathological procedure and embedded in paraffin. Approximately four-micron thick sections were cut and stained with haematoxylin & eosin and haematoxylin & van Gieson.

3:9 Histopathological analysis of bone marrow biopsies

The tumor tissue as well as the adjacent stroma and bone reaction may vary in quantity and quality in the BMBs containing metastatic tumor. The aim of the microscopical analysis of the metastatic tumor tissue was to establish:

- The relation between morphological findings in the BMB and the primary mammary tumors,
- Whether different morphological patterns were present within the same biopsy material, and
- whether prognostic information could be obtained from an analysis of the microscopic pattern.

In the first series of 47 metastatic lesions revealed by BMB, the 39 available primary mammary carcinomas were classified according to McDiwitt and Steward (15). Furthermore, in this series the consistency (morphological variation within the biopsy) was registered with respect to three basic morphologic observations, namely, the mode of tumor growth, the stroma reaction and the bone reaction (Paper III). In the series of 78 metastatic lesions (Paper V) the microscopical analysis also comprised a semiquantitative registration of eight different morphological variables performed in order to evaluate its prognostic value.

3:10 Statistical analysis

The death risk and cumulative survival of the patients were determined and calculated according to Mantel (31). For analysis of correlations between morphological variables and survival Pitman's non-parametric test was used (6).

4 RESULTS

4:1 Incidence of bone marrow metastases in the posterior iliac crest in patients with operable tumors stage I–III

Of the 415 patients with stage I–III tumors the BMB was positive in 30 and negative in 385. Table 7 shows the incidence of bone marrow metastases in relation to the tumor stage and time of investigation. The BMB was positive in 4 out of 103 patients (4 %) at the time of initial diagnosis, 1 in stage I, 3 in stage II and none in stage III. In the follow-up group the BMB was positive in 26 out of 312 patients (8 %), 2 in stage I, 13 in stage II and 11 in stage III.

CLINICAL STAGE	AT THE TIME OF INITIAL DIAGNOSIS		IN FOLLOW-UP $\frac{1}{2}$ – 6 YEARS	
	No OF PTS	No WITH BMB pos	No OF PTS	No WITH BMB pos
I	18	1	42	2
II	65	3	182	13
III	20	0	88	11
TOTAL	103	4	312	26

Table 7. Metastases in bone marrow biopsies from patients with breast cancer stage I–III

The correlation between clinical stage I–III operable breast cancer and BMB findings was not statistically significant ($p > 0.10$).

The results in the same patients studied with respect to the primary tumor location are shown in Table 8.

PRIMARY TUMOR LOCATION	CLINICAL STAGE	No. OF PTS	TOTAL	No. WITH BMB pos	TOTAL
CENTRAL	I	1	49	0	17
	II	21		8	
	III	28		9	
INNER	I	14	114	0	3
	II	78		2	
	III	22		1	
OUTER	I	45	252	3	10
	II	148		6	
	III	59		1	
TOTAL			415		30

Table 8. Metastases in bone marrow biopsies from patients with breast cancer, operable tumors in relation to the primary tumor location and clinical stage

The BMB was positive in 17 out of 49 patients (35 %) with central tumor location, in 3 out of 114 patients (3 %) with tumors in the inner half of the breast and in 10 of 252 patients (4 %) with tumors in the outer half of the breast.

The proportion of BMB positive findings was significantly higher ($p < 0.0001$) among patients with central tumors than among those with non-central tumor location.

Bone marrow involvement in relation to the hormonal status of the patients in stage I–III is demonstrated in Table 9. The BMB was positive in 10 out of 154 premenopausal patients (7 %) and in 20 out of 261 postmenopausal (8 %) patients.

CLINICAL STAGE	MENOPAUSAL STATUS	No. OF PTS	No. WITH BMB pos.
I	PRE	18	1
	POST	42	2
II	PRE	96	6
	POST	151	10
III	PRE	40	3
	POST	68	8
TOTAL	PRE	154	10
	POST	261	20

Table 9. Metastases in bone marrow biopsies from patients with breast cancer, operable tumors in relation to hormonal (menopausal) status

There was no significant correlation between menopausal status and BMB findings ($p > 0.20$).

The 415 patients operated upon were studied with respect to the size of the primary tumor (Table 10). The incidence of positive BMB increased with the size of the primary tumor. It was 2 % (2/86) in the group with tumor smaller than 2 cm, 6 % (15/253) in the group with 2–5 cm tumors and 17 % (13/76) in the group with tumors measuring 5–7 cm.

There was a significant correlation between tumor size and BMB findings ($p < 0.001$).

Out of 345 patients investigated, 146 had histophatologically proved axillary metastases (Table 11). Eighteen of these 146 patients (12 %) had positive BMB. Conversely, only 7 out of 199 patients (3.5 %) with histopathologically negative axillary nodes had positive BMB.

TUMOR SIZE MEASURED IN OPERATION SPECIMENS	No. OF PTS	No. WITH BMB pos
< 2 cm	86	2
2–5 cm	253	15
5–7 cm	76	13
TOTAL	415	30

Table 10. Metastases in bone marrow biopsies from patients with breast cancer, operable tumors in relation to tumor size

AXILLARY NODE HISTOPATHOLOGY	No. OF PTS	No WITH BMB pos
NEGATIVE	199	7
POSITIVE	146	18
NOT EXPLORED	70	5
TOTAL	415	30

Table 11. Metastases in bone marrow biopsies from patients with brest cancer, operable tumors in relation to axillary node histopathology

There was a significant correlation between axillary node histopathology and BMB findings ($p<0.01$).

Of the 415 patients with stage I–III tumors, 180 were related to BSC. The BSC was judged to be positive in 88, negative in 73 and doubtful in 19 patients.

The BMB was positive in 22 out of 88 patients with positive BSC, 3 in stage I, 11 in stage II and 8 in stage III. BMB revealed metastases in 6 out of 73 BSC-negative patients (in 3 patients with stage II and in 3 with stage III tumors), Table 12.

n=415

STAGE		I n=60		II n=247		III n=108	
PATIENTS WITH		BMB pos.	BMB neg.	BMB pos.	BMB neg.	BMB pos.	BMB neg.
BSC	POSITIVE	3	11	11	32	8	23
	NEGATIVE	0	10	3	40	3	17
	DOUBTFUL	0	3	0	11	0	5
	NOT DONE	0	33	2	148	0	52

Table 12. Correlation between bone scintigraphy (BSC) and bone marrow biopsy (BMB) in patients with breast cancer stage I–III

4:2 Incidence of bone marrow metastases in the posterior iliac crest in patients with stage IV tumors

Table 13 shows the results of BMB and BSC in 207 patients with stage IV tumors. The BMB was positive in 59 and negative in 148 patients. Positive BMB findings in patients with positive BSC were obtained in 29 out of 73 patients with osseous metastases only, 6 out of 24 patients with non-osseous metastases and 4

n=207

TYPE OF METASTASES		osseous n=96		non-osseous n=89		mixed n=22	
PATIENTS WITH		BMB pos.	BMB neg.	BMB pos.	BMB neg.	BMB pos.	BMB neg.
BSC	POSITIVE	29	44	6	18	4	10
	NEGATIVE	3	2	1	28	1	2
	DOUBTFUL	2	1	0	1	0	0
	NOT DONE	8	7	1	33	4	2

Table 13. Correlation between bone scintigraphy (BSC) and bone marrow biopsy (BMB) in patients with breast cancer stage IV

out of 14 patients with mixed metastases. Altogether BMB confirmed 35 % (39/111) of the bone metastases revealed by BSC. Moreover, BMB revealed metastases in 5 out of 37 BSC negative patients with stage IV carcinoma.

4:2.1 Peripheral blood levels in stage IV tumors

Two hundred and seven patients in stage IV were included. No prior chemotherapy had been given at the time of peripheral blood investigation.

The laboratory's normal limits for B-Haemoglobin were 116–149 g/l, for WBC 3.5–9.0 and for platelets 150–350 x 10^9/l.

Three peripheral blood smears were prepared, usually at the same time as BMB. The May-Grünwald-Giemsa-stained blood smears were studied under a light-microscope for determination of the percentage of neutrophils, basophils, eosinophils, monocytes and lymphocytes. The total lymphocyte and monocyte counts were measured by multiplying the percentage by the WBC counts. The normal limits for lymphocytes were 0.87–3.15 and for monocytes 0.17–0.72 x 10^9/l.

Total B-Hb, WBC, neutrophils, basophils and eosinophils were within the normal limits. A tendency towards lymphopenia was found in 54 patients, 20 of whom were BMB-positive for tumor cells. There was a tendency towards monocytosis in 20 individuals, 7 of whom had BMB metastases. Both abnormalities (lymphopenia and monocytosis) were present in 6 patients with positive BMB. Two patients had thrombocytosis (360 and 485 x 10^9/l respectively), neither of them had bone marrow metastases. Thrombocytopenia was encountered in 26 patients, 11 of whom had marrow involvement.

Primitive myeloid elements were described in 3 patients, all of whom had bone marrow metastases.

The relationships between the haematological abnormalities and BMB findings are summarized in Tables 14 and 15.

There was no significant correlation between BMB findings and abnormal peripheral blood levels ($p > 0.20$).

Serum electrolytes and the results of liver function tests were within the normal limits in all patients.

4:3 Histopathology of bone marrow metastases

The comparison of the microscopic findings in the metastases with the primary mammary tumors revealed that 61% of the metastases of mammary carcinoma with productive fibrosis had a compact mode of growth and had elicited some degree of collagen fibrosis and new bone formation. Of this tumor type, 20 % were osteodestructive and the remaining 19 % exhibited other morphological patterns.

		LYMPHOCYTES		MONOCYTES		PLATELETS		PRIMITIVE
	No. of pts	0,30 – 0,87	> 3,15	0,08 – 0,17	0,72 – 1,02	60 – 150	> 350	ELEMENTS *
BMB pos.	59	20	0	3	7	11	2	3
BMB neg.	148	34	0	10	13	15	0	0
TOTAL	207	54	0	13	20	26	2	3

*) Promyeloblasts, metamyelocytes, normoblasts

Table 14. Histopathology of bone marrow biopsies (BMB) in relation to abnormal levels in peripheral blood in patients with breast cancer stage IV

	No. of pts	WBC	Lymphocytes	Monocytes	Platelets
BMB pos.	59	4,8 ± 1,6	1,06 ± 0,16	0,45 ± 0,11	206 ± 24
BMB neg.	148	5,3 ± 1,8	1,26 ± 0,24	0,45 ± 0,12	193 ± 27

(MEAN ± SE)

Table 15. Histopathology of bone marrow biopsies (BMB) in relation to WBC, lymphocyte, monocyte and platelet counts in patients with breast cancer stage IV

The histopathology in 6 cases of the metastatic non-scirrhous carcinoma was varied (Paper III). In the three cases of metastases from medullary carcinoma there was no influence on bone tissue, none or only a slight fibrotic reaction and there was growth in compact cellular infiltrates (see Fig. 5, Paper III). The two metastatic papillary carcinomas had induced new bone formation, marked fibrosis and solid cellular growth. The metastatic lobular carcinoma was osteolytic with a pronounced fibrotic reaction.

The consistency of the observed morphological variables in the individual BMB was high; i.e. for tumor cell growth, stroma- and bone reaction, a consistent pattern was observed in 90 %, 87 % and 81 % of the biopsies respectively. Altogether, the consistent pattern for these three variables was found in 74 % of the biopsies.

Regarding the prognostic value of single morphological traits in the metastatic lesion, a statistically significant correlation was found between the nuclear polymorphism and survival from the time of BMB. All other correlations between single morphological variables and survival were non-significant.

A multiple regression analysis of the studied morphological variables revealed that the most informative variables in bone marrow metastases were nuclear polymorphism, mode of tumor growth and tumor cellularity.

4:4 Prognostic significance of bone marrow metastases

The death risk, determined according to Mantel (31), in 622 patients examined by BMB was greater in BMB-positive than in BMB-negative patients ($p < 0.0000001$, Paper IV, Fig. 1). In contrast, no difference in death risk existed between patients with positive and negative BSC ($p > 0.25$, Paper IV, Fig. 2). Similar results were found for a subgroup of 207 patients who underwent BSC and BMB within 5 months (Paper IV, Tables 6 and 7).

5 GENERAL DISCUSSION

5:1 Bone marrow biopsy results in stage I–III tumors

It is not known when the bone marrow metastases appear in relation to the development of the primary tumor, but findings reported by Fischer et al., 1975 (18) indicate that by the time a clinical diagnosis of breast cancer is established, most patients have disseminated disease. In our study the incidence of BMB-revealed metastases was relatively low, increasing from 4 % (4/103) at the time of initial diagnosis to 8 % (26/312) during follow-up after 6 months – 6 years in stage I–III operable breast cancer (Papers III and IV). The lack of significant difference between the BMB-findings in clinical stages I–III may be explained by the limited diagnostic accuracy in the pre-treatment evaluation of the primary tumor and axillary node status by palpation.

Handley, 1972 (22) discovered that a central zone tumor location significantly influenced tumor invasion of the internal mammary nodes and was associated with a poor prognosis. On examining 342 patients who had undergone radical surgery, they found that 52 % of 108 patients with tumors in the inner hemisphere and 53 % of 187 with tumors in the outer hemisphere were alive at 10 years, but only 38 % of those with central tumors were still alive.

The central zone seems to be the most dangerous site for bone marrow spread. Our findings of bone marrow metastases in 35% (17/49) of patients with central tumor location, corresponding to 3 % (3/114) and 2 % (10/252) respectively in patients with inner zone and outer zone tumor location, support Handley's observation. However, one explanation for this may be that tumors in the central part of the breast are detected somewhat later than tumors in other parts of the breast and are therefore more advanced, as demonstrated in Table 8.

With regard to pre- and postmenopausal women, there was no significant difference in the incidence of positive BMB (6.4 %, 10/154 versus 7.6 %, 20/261). The same is true for other clinical variables such as the disease-free interval and survival, as emphasized by Cooper and co-workers 1979 (12).

Tumor size and axillary node histopathology are classically two of the basic elements of the pathological classification of breast cancer. The percentage of BMB revealing metastases increased with increasing tumor size from 2 % in patients with tumors < 2 cm in diameter to 17 % in patients with tumors of 5–7 cm in diameter (Table 10).

In patients with positive axillary node histopathology the proportion of BMB-positive individuals was 12 % (18/146), compared to only 4 % (7/199) among those with negative axillary node histopathology.

5:2 Bone marrow biopsy results in stage IV tumors

At autopsy, Johnston 1970 (27) found bone metastases in 57 % of cases of breast cancer and Meissner & Warren 1971 (32) found bone metastases in 49 %. In a clinical study, Ingle et al. 1978 (25) analysed 116 patients with primary and advanced breast cancer and correlated the results of BMB in the posterior iliac crest with those of radiographic examination and BSC. Positive BSC findings were obtained in 55 % of patients with positive radiograms and in 56 % of patients with positive BSC, but only in 4 % (1/24) of those in whom both the BSC and radiograms were normal. We found positive BMB results in 43 % (51/118), 35 % (39/111) and 7 % (7/101) respectively. Ingle and co-workers found more BMB positive results in patients with distant metastases (40 %) than we did (28.5 %). This may be due to differences in the selection of patients.

Cohen and co-workers, 1979 (10) found positive BMB in 26.3 % of 38 patients with disseminated breast cancer.

5:2.1 Peripheral blood levels in stage IV tumors

The low incidence of leukoerythroblastic blood picture (5 % (3/59)) in the patients with stage IV carcinoma can be explained by the clinical characteristics of these patients: most of them were examined at an early phase of the metastatic disease. Similar findings were reported by Ingle et al., 1978 (25).

The lack of hypercalcaemia and normal levels of serum alkaline phosphatase in patients with metastases in bone marrow is in agreement with observations of Pistenma and co-workers, 1975 (39) and Cohen & co-workers, 1979 (10).

5:3 Bone marrow biopsy – technical aspects

BMB has been known for years to be superior to conventional cytological bone marrow aspiration in the examination of patients with solid tumors. This is well-documented by Contreas et al., 1972 (11), Burkhardt, 1973 (8), Bearden et al., 1974 (2) and by Landys & Stenram, 1975 (Paper I).

BMB can be performed with different types of needles or trephines. Needles give fragile cylinders and substantially smaller volumes of bone marrow. Trephines with larger diameters cause greater trauma and are therefore not suitable for out-patient use. The drill or twist biopsy technique according to Jamshidi or Gidlund, or the punch technique using Radner's or Stavem's needles can only be used when the marrow is of normal density or osteolytic. In sclerotic bone marrow or compact bone tissue, the instruments have

a tendency to bend, the sample may be damaged by compression and the obtaining of sufficient marrow for histopathological investigation may be difficult or impossible. For that reason a new type of instrument, called the LM-trephine was developed and introduced at our department (Paper II). Closed BMB from the posterior iliac crest using the LM-trephine has the following advantages:

- the quantity and technical quality of the specimens are more satisfactory than with needle techniques, and
- the risk of complications when taking specimens from sclerotic, osteoporotic or destroyed bone is reduced.

5:4 Histopathology of bone marrow metastases

Bone metastases are usually described as being osteoblastic or osteolytic. Our analysis (Papers III and V) revealed variations in the tumor and surrounding tissue in marrow metastases. The most common pattern was a moderate or pronounced new bone formation accompanied by marked fibrosis in the metastatic lesions. Predominantly osteolytic lesions were observed in 19 % of the biopsies. These findings differ from observations on osseous metastases in radiographic materials reported by Sutherland et al., 1932 (47), Hellner, 1935 (23), Murray & Jacobsson, 1977 (36) and in an autopsy material described by Milch and Changus, 1956 (34), where the majority of the lesions have been reported to be osteolytic.

Milch and Changus (op. cit.) also emphasized that osteolysis was more common in autopsy materials. This suggests that the findings of predominantly osteolytic lesions in breast cancer patients may represent a later stage of metastatic development. The differences between the histopathology at autopsy as reported by Milch and Changus (op. cit.) and in biopsies might be explained by the fact that the biopsy material in our study was taken at an earlier phase of the disease, all patients with stage I–III and the majority of patients with stage IV carcinoma being symptomless. Single small clumps of malignant cells or metastases without reaction of the surrounding tissue probably represent the early stage of tumor spread in the bone marrow. The increased activity of the adjacent fibrous, non-tumorous stroma and trabecular bone results in fibrosis, with collagenous formation and/or new bone deposition. This represents a later phase in the development of metastasizing and may facilitate the scintigraphic or radiographic diagnosis owing to better demarcation and infallible visualisation of the metastases. From the morphological point of view, it seems to be very important to evaluate the basic parameters in metastatic tumor tissue in BMB such as nuclear polymorphism, tumor cellularity and mode of growth for predicting prognosis in metastatic breast cancer.

5:5 Prognostic significance of bone metastases revealed by bone scintigraphy

Conflicting results concerning the prognostic value of BSC analysis have been reported. Galasko, 1975 (20), Campbell et al., 1976 (9) and El-Dormieri et al., 1976 (16) emphasized the rationale for bone scanning in breast cancer and concluded that this technique proves an excellent prognostic index in the initial assessment of the disease. In contrast to these findings, there was no difference in survival between BSC-positive and BSC-negative patients in the studies of Blair, 1975 (4) and Davies et al., 1977 (14).

The diagnostic sensitivity of BSC varies with the isotope used. The best results were obtained with ^{99m}Tc stannous methylene diphosphonate, owing to faster urinary excretion, which lowers the background activity and increases the difference between bone and soft tissue activity. The age of the patient also influences the diagnostic specificity and accuracy of BSC with respect to malignancy. Statistically, women over 50 years and postmenopausal women have abnormal BSC more often than young and premenopausal women, according to Campbell et al., 1976 (9). Moreover, a positive BSC reveals not only changes in the metabolism in the metastatic focus itself but also in the surrounding tissues. Inflammatory or degenerative diseases in the bone may therefore give a positive BSC simulating malignancy. This conclusion is supported by the study of Sklaroff and Charkes, 1968 (44), who found carcinoma in 79 % of biopsies taken from BSC-positive skeletal foci, while 21 % remained microscopically negative.

5:6 Prognostic significance of bone marrow metastases revealed by bone marrow biopsy

Mendoza et al., 1969 (33) and Ingle et al., 1977 (24) studied the prognosis in patients with breast cancer with positive BMB. Ingle (op. cit.), compared 15 BMB-positive and 28 BMB-negative individuals. Mendoza (op. cit.), using aspiration bone marrow biopsy, compared 41 patients with positive BMB with 96 patients with negative biopsies. The data presented in both studies show no correlation to the stage of the disease or the topography of the metastatic spread. In both studies the conclusion was drawn that BMB lacked prognostic value. This is not in agreement with our findings. The differences may be due to differences in the method, selection of patients and the relatively small numbers of patients in the previous studies.

We suggest that the prognostic superiority of BMB may be due to the following factors:

- The histopathological verification of metastatic osseous lesions in the biopsy is more specific than a positive BSC.
- A positive tumor diagnosis, revealed by BMB within a very small tissue volume, probably presupposes a more extensive spread of cancer cells in the skeleton and other tissues, with a consequent higher mortality.

6 GENERAL SUMMARY

BMB from the left posterior iliac crest was performed as part of the routine diagnostic investigation in 622 women with primary or metastatic breast cancer. In 332 of the patients BSC was also carried out at the time of intial diagnosis or during follow-up. The prognostic significance of the BMB was compared with that of clinical and pathological stage and BSC.

BMB revealed metastases in 7 % (30/415) of patients with carcinoma in stage I–III and in 28 % (59/207) of patients with stage IV tumors.

Positive BMB findings were obtained in 12 % patients with axillary metastases, in 17 % of patients with primary tumors of more than 5 cm in diameter and in 35 % of patients with tumors in the central zone of the breast. Hormonal status did not influence the results of BMB. BMB revealed metastases in 31 % (61/199) of patients with positive BSC, in 10 % (11/110) of patients with negative BSC, and in 7 % (7/101) of patients in whom both radiography and BSC were negative.

Statistical evaluation showed that the patients with positive BMB had a significantly higher mortality than patients with negative BMB. There was no significant correlation between mortality and BSC-results (either positive or negative).

Morphological evaluation showed that nuclear polymorphism in bone marrow metastases was correlated to a highly unfavourable prognosis.

In patients with breast cancer BMB may establish the degree of generalisation when metastases has been detected and may also be helpful in the interpretation of equivocal radiographic and BSC results. Moreover, BMB is a useful means of obtaining information on bone marrow morphology before starting systemic treatment.

BMB is recommended for verification of bone marrow metastases from breast cancer in:

- Patients with primary tumors larger than 5 cm;
- Patients with tumors in the central zone of the breast;
- Patients with axillary node involvement;
- BSC-negative patients with stage II–IV carcinoma, and
- all BSC-positive patients.

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9 PAPERS

PAPER I

Scand J Haematol (1975) **15**, 104–108

Bone Marrow Biopsy of the Posterior Iliac Crest with Gidlund's Instrument in Malignant Diseases

KAROL LANDYS, M.D. & UNNE STENRAM, M.D.

Department of Radiation Therapy (Chief, M. Lindgren)
and
Department of Pathology (Chief, U. Stenram), University Hospital, Lund, Sweden

The bone marrow was examined on material obtained with the Gidlund trephine and with conventional cytologic bone marrow aspiration in 225 patients with malignant disease, 144 of whom had malignant lymphoma, and in 5 patients with haematological, non-malignant disease. With Gidlund's instrument, the malignant disease was found to engage the bone marrow in 35 cases as compared to 7 cases with needle biopsy. No complications were met with either technique. Bone marrow biopsy was routinely performed in the out-patient department.

Key words: bone marrow – biopsy trephine – Gidlund's instrument – malignant disease

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Correspondence to: Dr. Unne Stenram, Department of Pathology, University Hospital, S-221 85 Lund, Sweden

There are several different ways of sampling bone marrow tissue for microscopy: with needles, trephines or by open myelotomy.

It is the aim of this investigation to compare the results obtained with the Gidlund trephine and with conventional cytologic bone marrow aspiration in 225 patients with malignant disease. 5 patients with haematological, non-malignant disease were also studied.

MATERIALS AND METHODS

We have used Gidlund's instrument for bone marrow biopsy, manufactured by Astra, Sweden. It consists of a manually-operated boring trephine with a toothed point, a faceted and planed mandrin for given sample-length and a sample-remover. The depth of the boring is also controlled by an indicator in the form of a ring. (See Figure 1).

Local anesthesia is performed with 10 ml 1 % Xylocaine (Astra, Sweden) for the skin and surrounding tissues, and with 5 ml 2 % Xylocaine for the periosteum. A small incision is made in the skin, and the shortest distance between the skin and the posterior iliac crest is punctured directly from behind at the S-1 level. The compact lamina is then trephined with the faceted mandrin, whereupon the mandrin is changed for a shorter, planed one, and after a few turns a cylinder-shaped sample of bone marrow, 2.5 mm in diameter, is obtained.

The length of the sample can vary between 10 and 25 mm, and its weight between 50 and 120 mg. Several bone marrow samples can be

obtained by boring at different angles from the same trephination opening. The operation is concluded by controlling haemostasis in the opening and ordinary skin suture.

All the bone marrow biopsies were performed on the left side of the posterior iliac crest. One fourth of them were performed in the out-patient clinic.

The material was immediately put into freshly-mixed Zenker formalin solution using Marton's method, and fixed for 4 h, whereupon it was rinsed in 6.6 % formaldehyde and finally fixed in formaldehyde solution of the same concentration for about 20 h (Marton 1973).

Decalcifying was performed in New Decalc (Betlehem Trading, USA) for about 20 min and the sample was then treated with routine histological technique and stained with haemotoxylin-eosin and according to van Gieson.

Investigation of 225 oncological patients has been carried out at initial diagnosis. There were 53 patients with Hodgkin's disease, 91 with non-Hodgkin lymphoma, 38 with breast cancer in stages I–IV, where no metastases in the pelvis had been found by roentgenogram or scintigraphy, and 43 who had other solid, extramedullary tumours. The blood values of all of these patients were within normal limits.

We have also investigated 5 patients without known tumour who suffered from severe anaemia. Usual routine methods had not given any clear basis for diagnosis of these patients.

Conventional cytologic bone marrow aspiration was performed at the same time in the sternal manubrium and in the posterior iliac crest 1 cm from the site of biopsy with a Wiman needle with an outer diameter of 1.4 mm. The usual technique was employed, with needle puncture, and the smear was stained according to May-Grünwald-Giemsa.

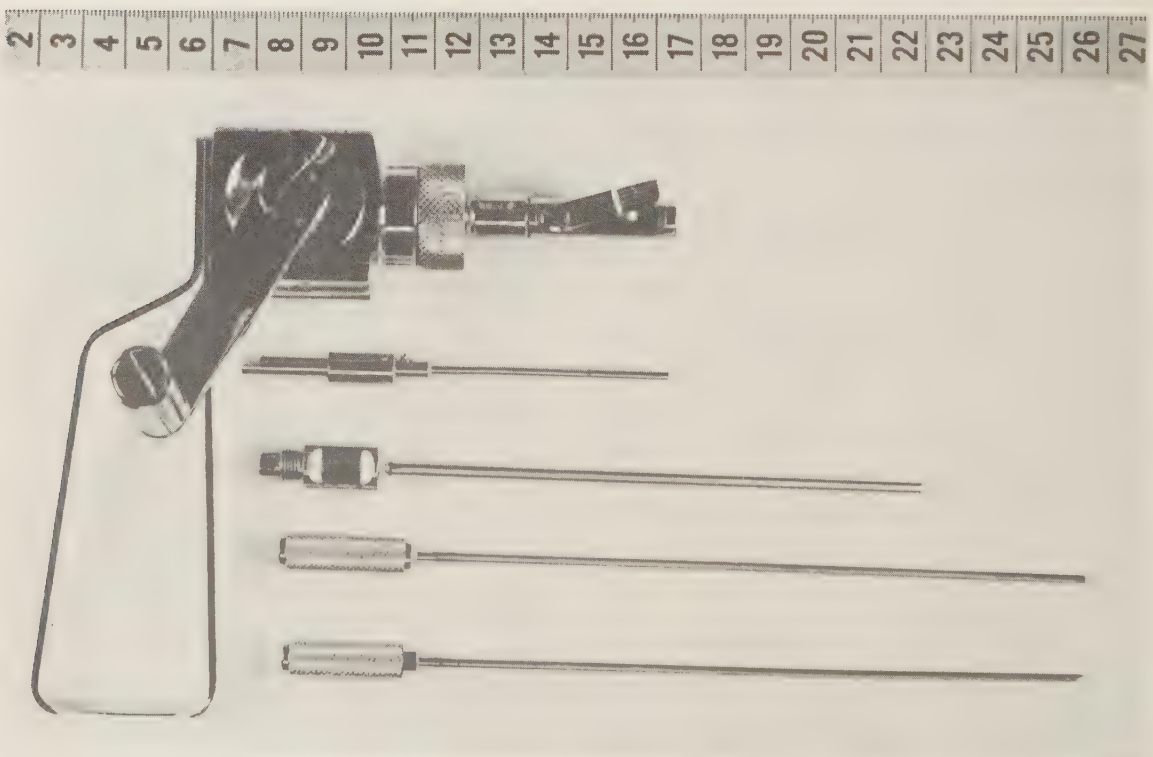

Figure 1. The Gidlund instrument for bone marrow biopsy.

TABLE I
Positive findings of bone marrow involvement

	Sternal and crest puncture	Gidlund's biopsy	Number of patients
Hodgkin's disease	1	4	53
Poorly differentiated lymphocytic lymphoma	4	11	31
Other non-Hodgkin lymphomas	1	4	60
Breast cancer	1	10	38
Other cancers	0	6	43
Total	7	35	225

RESULTS

As can be seen from Table I, malignant disease was found in the bone marrow much more often with the Gidlund instrument than with aspiration technique. Representative cases are illustrated in Figures 2 and 3. Besides engagement of the bone marrow with malignant disease, we also found other pathological changes, such as fibrosis, fibroblast proliferation and increased gathering of plasma cells or megakaryocytes.

2 cases of myelofibrosis were found in the group of 5 patients with severe anaemia. A third case showed epithelioid-cell granulomatosis (Figure 4). Follow up lead to the diagnosis of sarcoidosis.

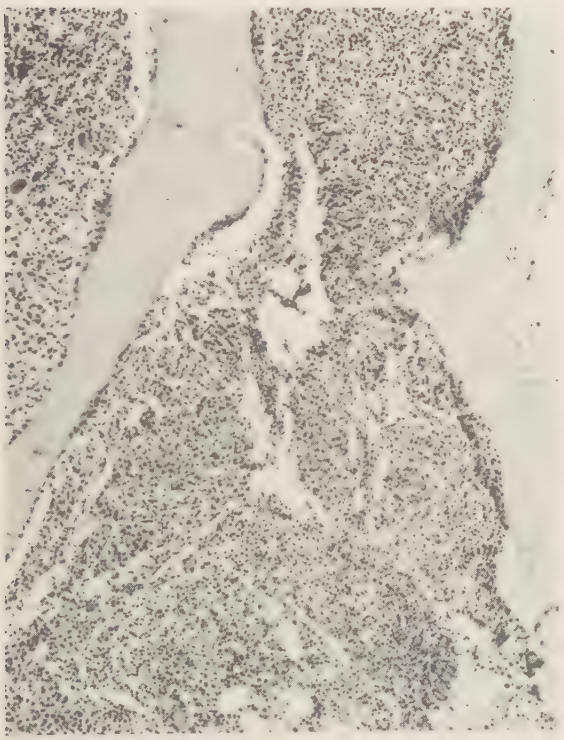

Figure 2. Bone marrow biopsy. Malignant lymphoma, diffuse, poorly differentiated lymphocytic type. There is a diffuse infiltration of lymphocytic cells. Haematoxylin-eosin. × 100

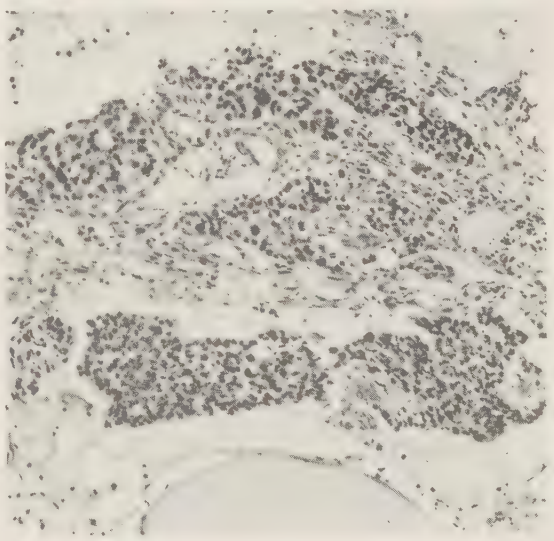

Figure 3. Bone marrow biopsy. Metastasis of carcinoma of the breast. There are several cords of cancer cells with dark nuclei. Haematoxylin-eosin. × 100

All of the investigations were free from complications. About one-third of the patients complained of soreness in the trephination site, which lasted about 24 h after the operation, but it could be satisfactorily relieved with analgetics administered orally.

DISCUSSION

The Gidlund instrument seems to have the following advantages:

The operations are easy to perform and can be done in the out-patient department. The incision in the skin is small compared to myelotomy using other instruments, and the periosteum need not be exposed.

Gidlund's instrument is manually operated, which allows a precise adjustment of the boring-speed and pressure to local conditions in the bone structure. This implies less risk for complications than when using an electrically driven boring trephine of the same calibre.

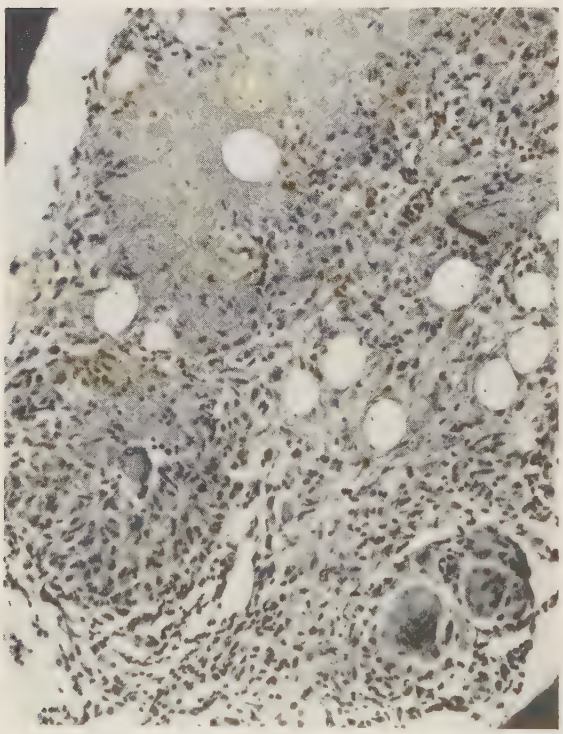

Figure 4. Bone marrow biopsy. Epithelioid cell granulomatosis. Several Langhans' giant cells are seen. A necrotic area is found in the upper left corner. van Gieson. × 160

Several bone marrow samples can easily be obtained from a single trephination opening and can thus give information on the bone marrow within a larger area. This gives considerably better diagnostic findings and material for more exhaustive histological techniques than conventional cytologic bone marrow aspiration.

The findings agree with those presented by Bearden et al (1974), who also found biopsies to give more positive findings than aspiration technique.

In our investigation the difference between the two methods was still larger, perhaps due to differences in the instruments used. Obviously Gidlund's instrument, as used here, is much superior to conventional cytologic bone marrow aspiration, and, in our material, showed no drawbacks as to morbidity.

Stavem (1967) and Helleberg-Rasmussen & Søndergaard-Petersen (1975) reported on the advantages with trephines, similar to that one used by us, but their papers do not contain any comparison of the trephine and the conventional cytologic bone marrow aspiration technique.

An advantage with the Gidlund instrument is the special indicator cannula, which makes it possible to insert the trephine into areas difficult to reach, with or without x-ray control, such as the vertebral bodies, the crista iliaca in obese or oedamatous patients and distant bones with local pathological alterations. The risk to puncture blood vessels, injure nerves etc. seems to be less than with other trephines.

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PAPER II

A New Trephine for Closed Bone Marrow Biopsy

Karl Landys

Department of Radiotherapy, Sahlgrenska sjukhuset, University of Göteborg, Göteborg, Sweden

Key Words. Bone marrow · LM-trephine biopsy · Osteoblastic metastases

Abstract. Our experience with a new trephine, called the LM-trephine, for carrying out bone marrow biopsy is reported. This trephine is larger and stronger than most previously described instruments. Two main problems in bone marrow biopsy, namely compression and difficulty in obtaining specimens from osteoblastic (or sclerotic) metastases are overcome by the special design of the LM-trephine tip. 310 out-patients with different tumour diseases were examined, and no complications were observed.

Microscopic examination of the bone marrow constitutes an essential oncological investigation. The demonstration of tumour cells in the bone marrow is regarded as evidence of generalized malignancy, with all the prognostic and therapeutic consequences it entails. The classical method of investigation comprises aspiration cytology of the sternum, spinous processes of the lumbar and thoracic vertebrae and the iliac crest with fine needles. This technique rapidly became a routine procedure in the investigation of plasmocytoma, and made important contributions to diagnoses in oncology and haematology. Several studies have shown that this technique may not be satisfactory for the diagnosis of marrow involvement in malignant lymphomas and metastatic malignancies like small-cell carcinoma of the lung, prostatic cancer, breast cancer, thyroid cancer and hypernephroma. Among the many workers contributing to the technical and diagnostic development of bone marrow biopsy, *Dameshek* [2], *Silverman* [10], *Burkhard* [1], *Radner* [8], *Landys and Stenram* [6], *Helleberg-Rasmussen and Søndergaard-Petersen* [4], *Stavem* [11], *Miller and Dennis* [7], *Jamshidi et al.* [5] and *Fornasier and Vilaghy* [3] deserve special mentioning.

Material and Methods

Between 1973 and 1977 we used Gidlund's instrument for bone marrow biopsies in 800 out-patients with breast cancer, malignant lymphomas and other tumours [6, 9]. In 1977 a new type of instrument, called the LM-trephine, was intro-

duced. It was developed at our department and is manufactured by and available from Änggårdens Instrumentverkstad, Sahlgrenska sjukhuset, Göteborg, Sweden.

The instrument consists of a hand-powered trephine, a faceted mandril and a specimen extractor (fig. 1). The internal diameter of the trephine is 4 mm, the external diameter 5.1 mm and the total length 162 mm (fig. 1). The tip of the trephine has ten teeth. Every other tooth is bent inwards 5°, which facilitates removal of the marrow specimen.

Biopsy Technique for Specimens from the Posterior Iliac Crest

Normally, the patient sits on a 40- to 50-cm-high stool and leans forward with his arms on a table. But the investigation can also be carried out with the patient lying on his stomach or side.

Under local anaesthesia with 8–10 ml of 2% Xylocaine (Astra), a 0.5-cm skin incision is made over the posterior iliac crest, about 5 cm from the midline. The bone is punctured at the level of the first sacral vertebra, about 7 cm below the top of the iliac crest. The lamina compacta is perforated with the faceted mandril inserted in the trephine. The faceted mandril is then removed and the trephine slowly advanced into the bone marrow with clockwise-counterclockwise motions until adequate marrow is obtained. The trephine is then withdrawn 2–3 mm and rotated, and its tip tilted at a slightly different angle, thus cutting the specimen inside the trephine free from the bone marrow outside the trephine. In the next phase, the trephine is slowly withdrawn with alternating rotatory motions. The specimen is removed through the opposite end of the trephine, as recommended by *Jamshidi et al.* [5]. The biopsy can be performed at right angles or oblique to the surface of the body (fig. 2).

In sclerotic bone, bending of the trephine must be avoided. In such cases the biopsy has to be performed straight through the bone marrow cavity to the opposite lamina compacta in order to obtain an adequate specimen.

The diameter of the specimen is 4 mm. The length may vary between 10 and 60 mm and the weight between 50 and 350 mg (fig. 3).

After control of haemostasis in the puncture channel, the incision is closed with a single skin suture. The biopsies are taken without premedication as an out-patient procedure. Every patient is examined 10 days after the biopsy.

Results

310 patients underwent bone marrow biopsies with the LM-trephine technique (140 patients with breast cancer, 95 with lymphomas, 35 with prostatic cancer and 40 with other tumours). 66 patients complained of tenderness at the site of the trephination for up to 6–12 h after the procedure. The pain could be satisfactorily relieved with oral analgesics, e.g. paracetamol. 36 patients had thrombocytopenia, with platelet counts varying between 35 and 97 $\times$ 10^9/l. Complications such as bleeding or infection did not occur.

Discussion

Closed bone marrow biopsy can be performed with different types of needles or trephines. Each method has its advantages and disadvantages and places certain demands on the patient and on the person carrying out the procedure. The aim of all methods is to obtain sufficient marrow for histological investigation (fig. 4, 5). In hyperostosis or pathologically altered bone, the closed biopsy technique may be difficult or impossible. The drill or twist biopsy technique according to Jamshidi or Gidlund, or the punch technique using Radner's or Stavem's needles can only be used when the marrow is of normal density or osteolytic. In sclerotic bone marrow or normal compact bone tissue, the instruments have a tendency to bend and the sample may be damaged by compression (fig. 6). This is well known for sclerotic osseous metastases of prostatic cancer, but can even happen in bone-producing osseous metastases of breast cancer [9] and radiation-induced fibrosis in the bone.

Fig. 1

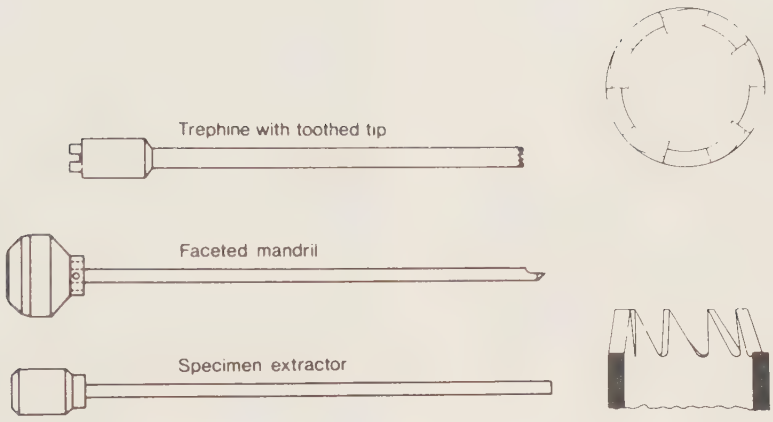

Trephine with toothed tip
Faceted mandril
Specimen extractor

Fig. 2

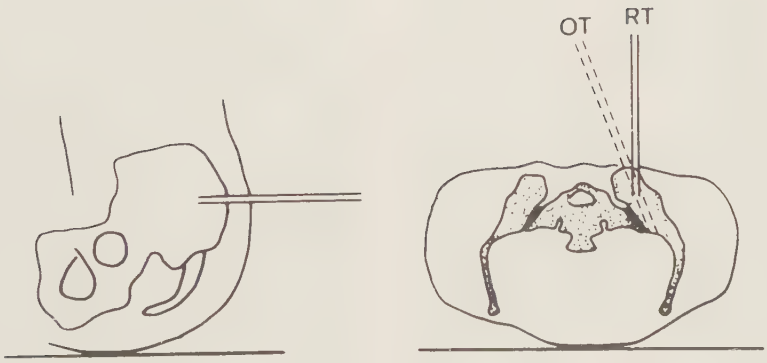

OT
RT

Fig. 3

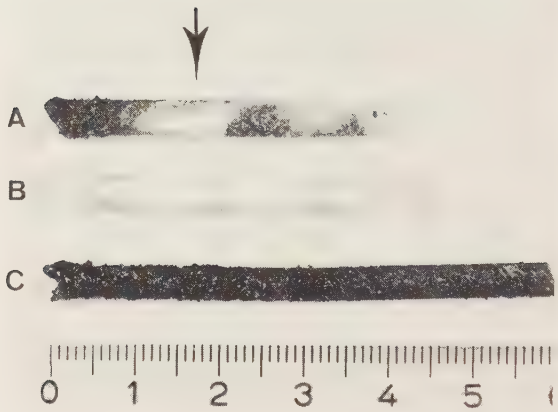


Fig. 4

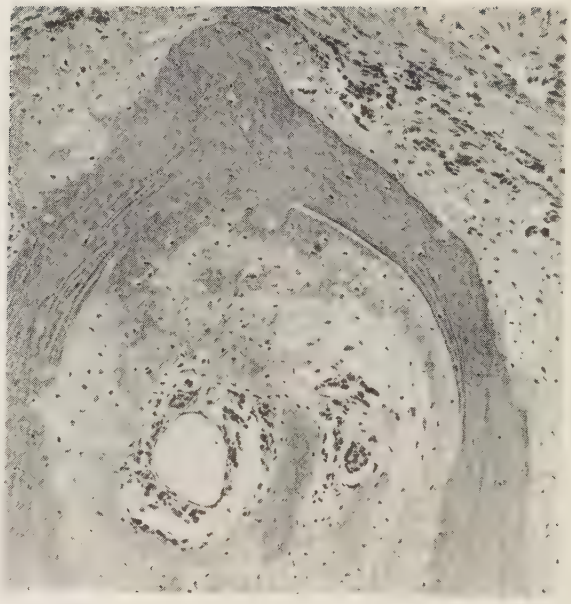


Fig. 5

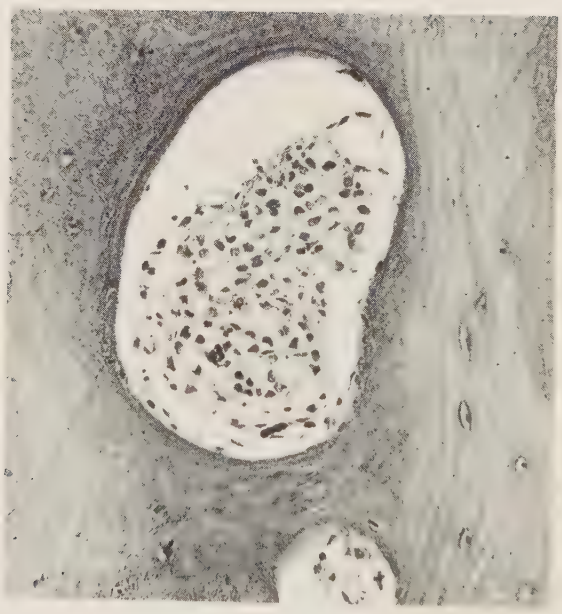
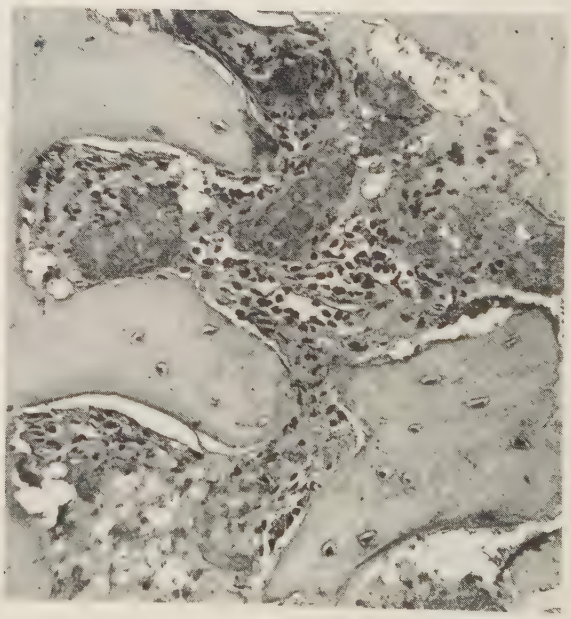


Fig. 6



With the LM-trephine biopsy technique, specimens of good quality could be obtained from all patients investigated, even from those with sclerotic bone metastases. Compression and crushing of specimens could be avoided thanks to the special design of the toothed trephine tip. The method ensures the patient minimal trauma, and yields sufficiently large bone and marrow specimens in their intact state, as demonstrated in figures 4 and 5. Histological specimens of this type and quality are not obtainable by the conventional needle biopsy technique.

The LM-trephine is larger, stronger and more effective in obtaining biopsies in sclerotic bone and bone marrow than Jamshidi's needle.

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Karol Landys, MD,
Department of Radiotherapy,
University of Göteborg, Sahlgrenska sjukhuset,
S–413 45 Göteborg (Sweden)

Fig. 1. The LM-trephine.

Fig. 2. Biopsy technique from the posterior iliac crest performed at a right angle (RT) or oblique (OT) to the body.

Fig. 3. Specimens of bone marrow obtained with the LM-trephine. A = 38-mm-long sample of inhomogeneous marrow tih a sclerotic part (arrow). B = 40-mm-long sample of compact sclerotic marrow in a patient with X-ray-treated, metastasis of breast cancer in the posterior iliac crest. C = 60-mm-long sample of homogeneous, macroscopically normal red marrow.

Fig. 4. Osteoblastic metastasis of breast cancer with newly formed woven bone. Focus of tumour cells around the vessel. Pronounced fibrosis at top right. Obtained with the LM-trephine. Haematoxylin and van Gieson. × 80.

Fig. 5. Osteoblastic metastasis of ductal invasive breast carcinoma. Small focus of tumour seen within dense bony tissue. Obtained with the LM-trephine. Van Gieson. × 200.

Fig. 6. Compression of the sample with artefact (crush) on the marrow sample obtained with Gidlund's instrument. HE. × 200.

PAPER III

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INCIDENCE AND HISTOPATHOLOGY OF METASTASES OF MAMMARY CARCINOMA IN BIOPSIES FROM THE POSTERIOR ILIAC CREST

B. Ridell, MD, and K. Landys, MD

Bone marrow biopsies were taken from the posterior iliac crest in 532 women with unilateral breast cancer. Metastatic tumors were found in 10% of the biopsies. In a group with negative radiological examinations of the skeleton, the incidence of positive bone marrow biopsies was 1.6%. In a group with radiologically detectable metastases in the skeleton 28% of the biopsies were positive. In the latter group 43 out of 45 individuals with positive biopsies had negative x-rays of the pelvis. Histopathologically, 19% of the metastatic tumors were osteolytic, 65% were osteoblastic and 16% did not influence the bone structure. The fibrous reaction in and around the bone marrow tumors was similar to that found in the primary tumor. In 74% the morphological pattern was consistent throughout the biopsy, whereas in 26% the morphology was different in different parts of the biopsy. No specific histopathology was observed in the individuals with negative radiological examinations of the skeleton. Bone marrow biopsy of the posterior iliac crest does not seem to be helpful as a routine method in the initial staging of mammary carcinoma but may contribute to establish the degree of tumor spread in individuals with positive or suspicious x-ray of the skeleton.

Cancer **44:1782–1788, 1979.**

Staging of malignant disease requires several diagnostic procedures. Examination of the skeleton, being one of the major locations for metastatic lesions from malignant epithelial tumors, is preferably performed with x-ray or scintigraphic techniques. However, a useful complementary measure for confirmation of metastatic lesions is bone marrow biopsy. Several authors have described the tumor morphology in metastatic lesions from mammary carcinoma at necropsy, but reports on the morphology in biopsies are sparse. In this study the value of bone marrow biopsy and the histopathology of metastatic mammary carcinoma is described and discussed in relation to x-ray findings.

From the Departments of Pathology I and Radiotherapy, Sahlgrenska sjukhuset, University of Göteborg, Göteborg, Sweden.

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Address for reprints: Karol Landys, MD, Dept. of Radiotherapy, Sahlgrenska sjukhuset, S-413 45 Göteborg, Sweden.

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Materials and Methods

Bone marrow biopsies were taken in 532 women aged between 29 and 85 years with unilateral breast cancer. The tumor disease was staged clinically according to the TNM system developed by UICC and AJC, Geneva 1972 (18). The patients were divided into 3 groups, according to the clinical stage, as follows:

Group 1: 103 patients with stage I–III breast cancer investigated at the time of initial diagnosis.

Group 2: 270 patients with stage I–III breast cancer who had undergone radical or modified radical surgery or surgery + radiotherapy and had not suffered clinical recurrence ½–3 years after treatment.

Group 3: 159 patients with stage IV breast cancer and skeletal metastases diagnosed by x-ray. The patients in this group received combined chemotherapy ad modum COOPER, modified as follows:

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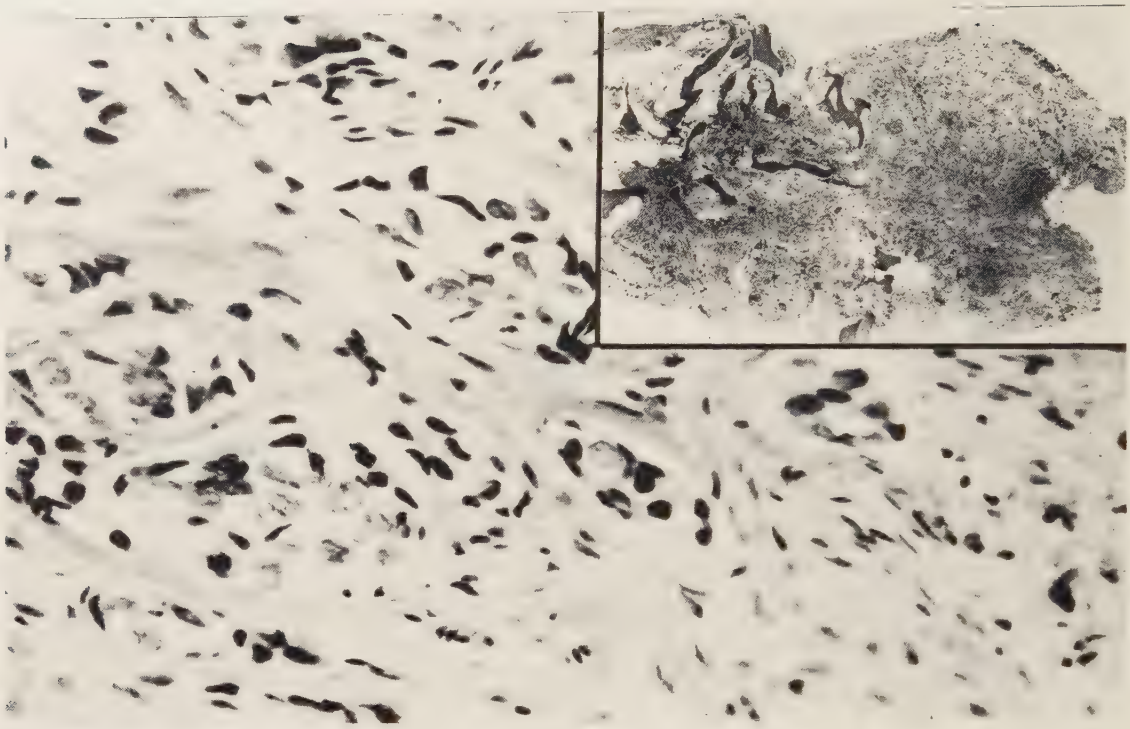

FIG. 1. Metastatic tumor with a compact growth and a pronounced fibrous stroma reaction. Biopsy from an osteolytic metastasis with small areas of new bone formation (H & v. Gieson ×500; inset ×25).

Oncovin 1 mg + Methotrexate 25 mg + 5-Fluorouracil 500 mg + Cyclophosphamide 500 mg iv once a week. 16 patients also received Primobolan Depot® (Metenolon-heptanoat, Schering AG), 100 mg im once a week.

Radiological examinations of the lungs, thoracal and lumbar column and pelvis were carried out in all patients in accordance with routine diagnostic procedures.

The biopsies were taken from the left posterior iliac crest using the Gidlund instrument.[7] The specimens were fixed in neutral 6% formaldehyde solution, decalcified in 2.5% nitric acid and embedded in paraffin, and approximately 4 microns thick sections were cut. The sections were stained with H & E and H & van Gieson.

The available primary tumors from individuals with metastatic lesions in the biopsies were reexamined and classified histologically according to the method of Stewart and McDivitt.[10]

In the bone marrow biopsies the amount of tumor tissue was subjectively graded as less than 25%, 25–50%, 50–75% or more than 75% of the section area. The influence of the tumor on bone tissue was registered on a 5-point scale, as follows: (1) with destruction of bone tissue (Fig. 1), (2) without influence on bone tissue (Fig. 2), (3) with slight, (4) with moderate or (5) with pronounced new bone formation (Fig. 2).

The mode of growth of the tumor was registered as compact (Fig. 1) or loose infiltrates (Fig. 3). The fibrous stroma reaction was registered as none, slight (Fig. 3) or pronounced (Fig. 1). The consistency for three of these individual variables, namely the type of bone reaction, the type of fibrous reaction and the density of tumor cell infiltrates, was registered, *i.e.*, whether there were great differences of the observed variables in different parts of the biopsy material.

RESULTS

The numbers of individuals in the different clinical groups with metastases in the iliac bone marrow are shown in Tables 1A and 1B. The incidence of metastasis in the whole material was 10%. The technical quality of four biopsies in group 3 permitted only a

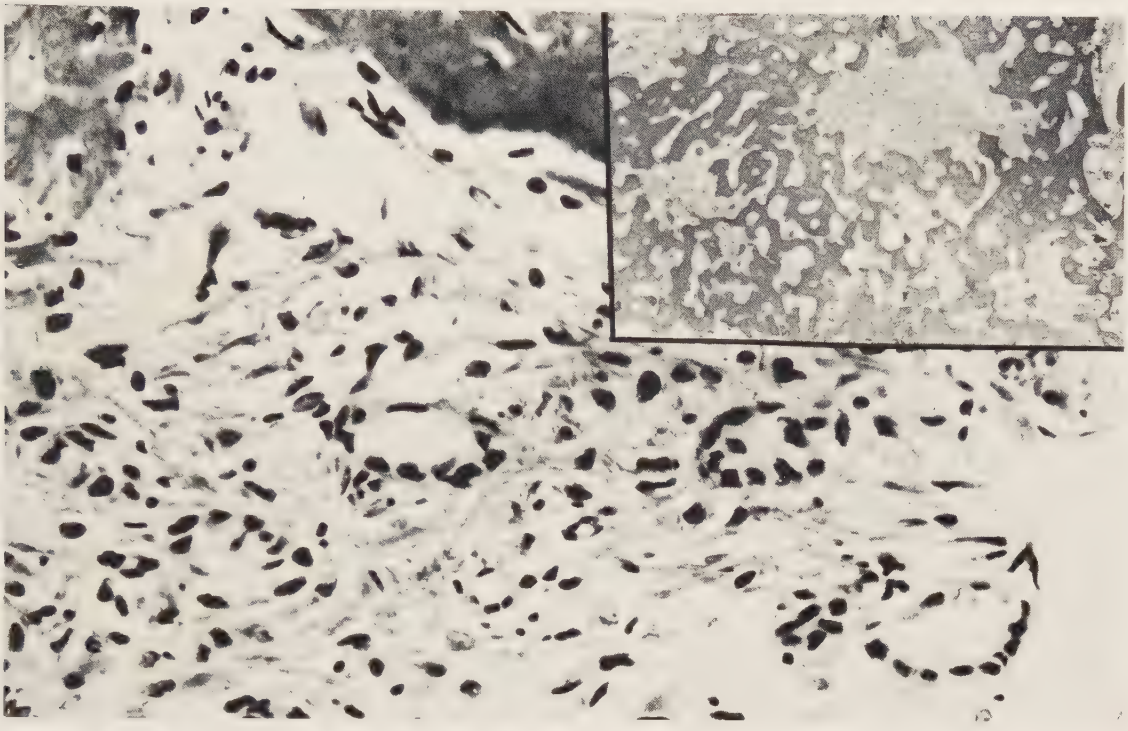

FIG. 2. Metastatic tumor with duct formation and a well-developed fibrous stroma. Biopsy from a metastasis with prominent new bone formation (H & v. Gieson ×500; inset ×25).

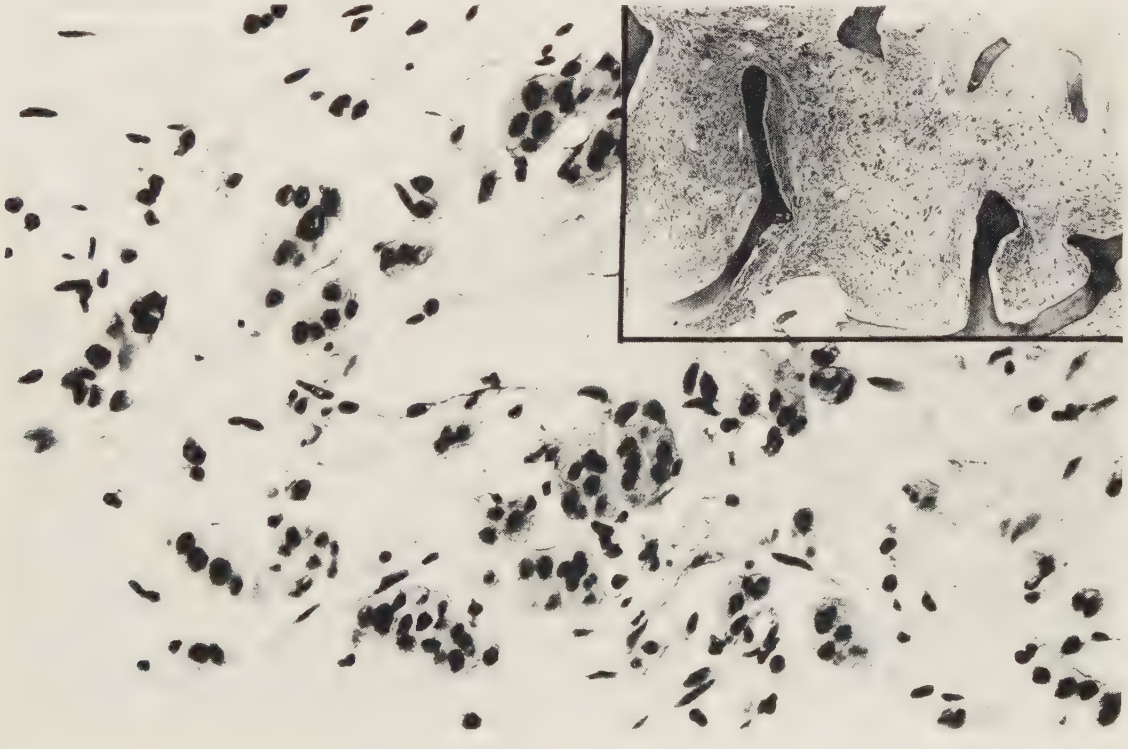

FIG. 3. Metastatic tumor growing in a loose pattern with a sparse stroma. Biopsy from a metastatic tumor not affecting the bone structure. (H & v. Gieson ×500; inset ×25).

TABLE 1A. No. of Positive Biopsies in Patients of Different Clinical Stages

Stage	No. of patients	No. of metastases
I	58	1
II	232	3
III	83	2
IV	159	45
TOTAL	532	51

qualitative diagnosis of metastatic carcinoma, without further morphological analysis.

Two of the 51 positive iliac crest biopsies came from individuals with radiologically detectable metastases in the pelvis. The other 49 individuals had negative x-rays of the pelvis.

The estimated amount of tumor tissue in the remaining 47 biopsies was less than 25% in 12 cases, 25–50% in 5 cases, 50–75% in 6 cases and more than 75% in 24 cases. Ten of the 17 biopsies with less than 50% tumor tissue had one or few focal lesions (Fig. 4), the other seven metastases were located in the periphery of the biopsy and might well have been part of a larger lesion.

In the entire biopsy material 19% of the tumors were bone-destructive, 16% did not affect the bone architecture and in 65% of the lesions some degree of new bone formation occurred (Table 3).

The degree of fibrous reaction and the type of tumor cell growth are shown in Table 4. The majority of the metastatic tumors (62%) induced a marked fibrosis, while in 30% there was slight fibrosis and in the remaining 8% no fibrosis was registered. 81% of the tumors grew with a compact and 19% with a loose infiltration.

The consistency of the observed morphological variables is shown in Table 5. In most of the cases in which there was new bone formation without bone destruction the pattern was similar in different parts of the biopsy material. In those tumors in which bone destruction was evident the pattern was different in different parts of the biopsy materials. As regards the stroma reaction in the metastatic lesion, the pattern was different in 5 out of 47 biopsies. The cell density of the tumor tissue differed in different parts of the biopsy material in 5 out of 47 biopsies. The overall number of biopsies with a consistent pattern with respect to bone reaction, stroma reaction or cell density was 46 (74%).

Of the 51 individuals with metastasis, ten had had inoperable mammary tumors. In these patients the diagnosis of mammary carcinoma was based on cytological examination of aspirated tumor material. 39 of the 41 mastectomy specimens were available and the histopathological classification of these tumors is shown in Table 2. The histopathology of the metastasis from nonscirrhous carcinoma was variable. Thus, the three metastases from medullary carcinoma had no influence on bone tissue, no or only a slight fibrotic stroma reaction and were growing in compact cellular infiltrates (Fig. 5). The two metastatic papillary carcinomas had induced new bone formation and marked fibrosis and the cellular growth was solid. The metastatic lobular carcinoma was osteolytic, with a pronounced fibrotic reaction. On average, the fibrotic reaction in the metastatic lesions was of the same degree as seen in the primary tumors, both for the scirrhous and for the nonscirrhous carcinomas.

TABLE 1B. No. of Positive Biopsies in Different Clinical Groups (see text)

Group	Stage	No. of patients	No. of metastases
1	I–III initial diagnosis	103	4
2	I–III follow-up	270	2
3	IV skeletal metastases on X-ray	159	45
TOTAL		532	51

DISCUSSION

Several authors have established the superiority of bone marrow biopsies compared with bone marrow aspirations for detection of metastases of malignant epithelial tumors in the bone marrow.[1,3,6,12,14,16] Since bone marrow metastasis may occur anywhere in the skeleton, and only a few small sites are available for biopsy-taking, x-ray or scintigraphic examination will reveal more metastatic lesions.[5] However, in cases in which these methods do not give conclusive results, or if

TABLE 2. Histological Type of Primary Tumor in 39 Individuals with Metastases in Biopsies

Ductal carcinoma with productive fibrosis (scirrhous carcinoma)	33
Medullary carcinoma	3
Papillary carcinoma	2
Lobular carcinoma	1

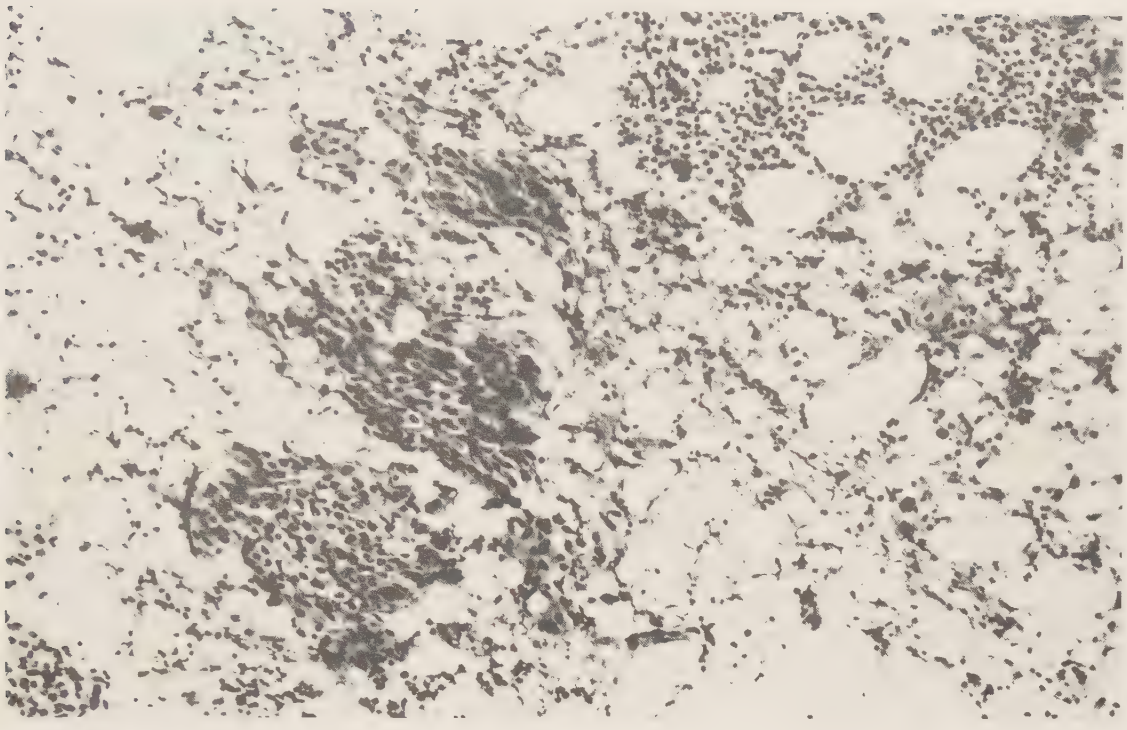

FIG. 4. Small metastatic foci in the bone marrow. No stroma reaction, degeneration of the bone marrow surrounding the metastasis. Preserved bone marrow in upper right corner (H & v. Gieson ×200).

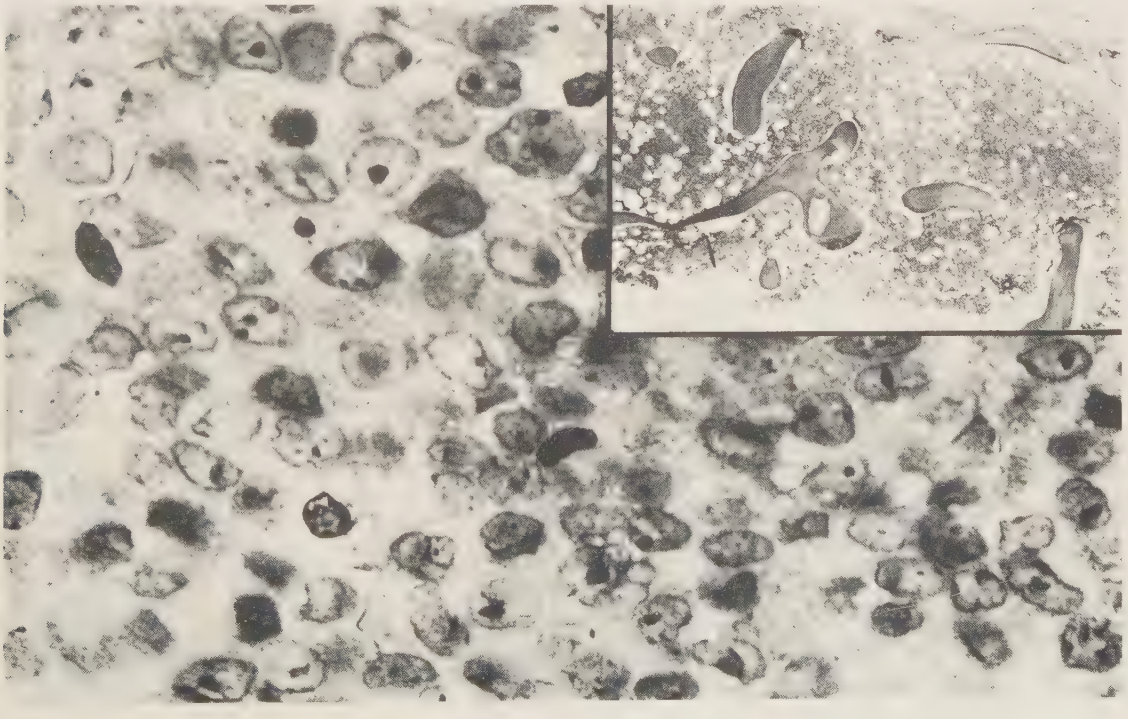

FIG. 5. Metastasis from a medullary carcinoma of the breast. There is no stroma reaction and the bone marrow fat is preserved within the tumor tissue (H & v. Gieson ×950; inset ×25).

TABLE 3. Bone Reaction in Biopsies with Different Amounts of Tumor Tissue

	Amount of tumor tissue	
	Less than 50%	Over 50%
Destruction of bone tissue	0	10
No reaction in bone tissue	5	5
Slight new bone formation	6	2
Moderate new bone formation	3	5
Pronounced new bone formation	1	10

the results of x-ray examination and scintigraphy are contradictory, a bone marrow biopsy may reveal metastasis.[8] In the present series there were six patients who had negative radiological examinations but in whom bone marrow metastases were found in biopsies. Slager and Reilly[16] reported 16 cases of bone marrow metastasis in patients with normal radiological bone surveys in a series of 410 malignancies of different types, and Singh *et al.*[14] reported bone marrow metastasis detected microscopically in 60% of subjects with malignant tumors with x-rays or bone scans considered to be normal. Ingle *et al.*[6] recently published results of bone marrow examination in mammary carcinoma. In their series 14% of patients with normal x-rays had metastases in biopsies. These figures are higher than the findings in the present series of mammary carcinoma, in which 1.6% of the patients with negative x-ray examinations of the skeleton had metastases in the bone marrow biopsies.

Several studies[5,13,15] have established the superiority of bone scan to assess skeletal metastases and in present series five of the six patients with biopsy-proven metastases and negative radiograms had a positive bone scan, the sixth one being negative.

Depending on the influence on bone tissue, metastases in the skeleton are usually described as being osteoblastic or osteolytic. In the present biopsy material the most common pattern was a moderate or pronounced new bone formation accompanied by marked fibrosis in the metastatic lesions. Predominantly osteolytic lesions, with destruction of bone tissue, were observed in 19% of the biopsies. These figures differ from observations on bone marrow metastases in radiological materials,[4,9,17] where the majority have been reported to be osteolytic[4,9,11] and also from studies on autopsy material[11] where osteolytic changes were more common.

TABLE 4. Degree of Fibrous Reaction and Type of Tumor Cell Growth

	Degree of stroma reaction		
	No fibrosis	Slight fibrosis	Pronounced fibrosis
Loose cellular growth	0	6	3
Compact cellular growth	4	8	26

In the biopsies which only partly consisted of tumor tissue, all patterns of bone reaction and fibrosis could be seen, but the dominant lesion was that of a compact cellular infiltrate with no or only a slight stroma reaction. It is possible that tumor metastases must attain a certain size or age before fibrosis develops. In most instances a metastasis from a scirrhous mammary carcinoma induced a prominent fibrosis in the bone marrow also. The number of nonscirrhous carcinomas studied was rather small but it seems likely that some medullary carcinomas of the breast lack the capacity to induce fibrosis in the metastasis in the bone marrow. Other investigators have considered that the metastasis in the skeleton differs in morphology at different times due to transformation from one type of metastasis to the others.[4]

TABLE 5. Consistency of Observed Morphological Variables in Individual Biopsies

	Bone reaction		
Consistent pattern in whole biopsy	Osteolysis	No bone reaction	New bone formation
Yes	0	7	31
No	9	0	0
	Fibrous reaction		
Consistent pattern in whole biopsy	No fibrosis	Slight fibrosis	Pronounced fibrosis
Yes	4	10	27
No	0	3	3
	Tumor cell growth		
Consistent pattern in whole biopsy	Loose cellular growth		Compact cellular growth
Yes	7		35
No	1		4

In the present material, 74% of the biopsies exhibited only one histological pattern. The differences between the histology in autopsy and biopsy material as well as the high consistency of the histopathology in the biopsies, might be explained by the fact that the biopsies are taken in an earlier phase of the disease. However, the possibility that the posterior iliac crest is a location in which predominantly osteoblastic metastases develop must also be considered. Only 2 of the 53 individuals with positive bone marrow biopsies had radiologically detectable metastases in the pelvis. Skeletal metastases must be at least 15–20 mm in diameter to be detected by x-ray.[2] However, not only the size but also the tissue reaction around the cancer cells may be of importance in this connection. Metastases with little or no reaction in the surrounding marrow and of predominantly osteoblastic type may be more difficult to detect on plain x-rays. In patients with breast cancer bone marrow biopsy may therefore provide a means of establishing the degree of generalization when metastasis has been detected radiographically and may also be helpful in the interpretation of equivocal x-ray results.

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PAPER IV

PROGNOSTIC VALUE OF BONE MARROW BIOPSY IN BREAST CANCER

By

Karl Landys

Department of Radiotherapy, Jubileumskliniken, Sahlgrenska sjukhuset, University of Göteborg, Göteborg, Sweden.

Supported by grants from the Jubilee Clinic Foundation for Cancer Research, Göteborg, Sweden.

Address for reprints: Karl Landys, M.D.
Department of Radiotherapy, Jubileumskliniken
Sahlgrenska sjukhuset, S-413 45 Göteborg, Sweden

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SUMMARY

Bone marrow biopsies (BMB) were performed on 622 women with a history of primary or metastatic breast cancer. In 332 of the patients bone scintigraphy (BSC) was also carried out at the time of initial diagnosis or during followup. The BMB revealed metastases in 22 of 88 patients in stage I - III with positive BSC and in 39 of 111 patients (35 %) in stage IV with positive BSC, totally in 61 of 199 patients (31 %). Moreover, the BMB was positive in 6 of 73 patients in stage I - III with negative BSC, and in 5 of 37 patients in stage IV with negative BSC, totally 11 of 110 patients (10 %).

Statistical evaluation showed that the patients with BMB revealing tumor had a significantly higher death risk ($p < 0.0000001$) than patients with negative BMB. No significant correlation was found in patients with positive or negative BSC. Similar results were also found for a subgroup of 207 patients who underwent BSC and BMB within 5 months.

BMB may therefore be indicated for verification of metastases and prediction of prognosis in all patients with positive BSC and in BSC-negative patients stage II - IV.

INTRODUCTION

It is generally accepted that the choice of treatment for breast cancer should be based on the clinical stage of the disease or, better, on the histopathological verified dissemination of the disease and grade of malignancy of the tumor. It is therefore an advantage if early and subclinical dissemination of the disease can be detected.

The early diagnosis of bone metastases has been considerably facilitated by the introduction of bone scintigraphy, which is more sensitive than radiographic examination of the skeleton (4). However, BSC does not seem to be a reliable technique for predicting the prognosis at the time of initial diagnosis (1, 4) and the value of BMB as a routine diagnostic procedure in patients with breast cancer is not known (9). In this study the BMB was correlated with the clinical stage, and BSC with respect to the prognosis in patients with breast cancer.

PATIENTS AND METHODS

Of 1950 patients with primary and 503 patients with metastatic breast carcinoma treated at the Jubileumskliniken between 1975 and 1979, 622 individuals had a BMB and 332 of these also had a BSC performed as part of the routine diagnostic work-up at the time of initial diagnosis or during followup 1/2 - 6 years after surgery. The mean age of the BMB patients was 59 years (range 27 - 85) and the mean age of the BSC patients was 58.5 years (range 27 - 83). Only outpatients were included in this study. The diagnostic work-up comprised a case history, physical examination and investigation of blood hemoglobin, white blood cells, platelets, peripheral blood smears, serum electrolytes, urinalysis and liver function tests.

The routine radiographic examination included the chest, thoracic and lumbar spine and pelvis.

The malignancy of all primary tumors was verified by microscopical examination. All patients were clinically staged according to the UICC-TNM classification 1972 (16).

Bone scintigraphy (BSC)

BSC was performed at the Division of Nuclear Medicine, Sahlgrenska sjukhuset, with a scintillation camera Pho/Gamma III HP (Searle) or Maxi camera II (General Electric), using a low energy, high resolution, parallel-hole collimator. The first 65 patients were examined with 99mTc pyrophosphate or polyphosphate and the remaining 244 patients with 99mTc stannous methylene diphosphonate (New England Nuclear). The routine BSC included the skull, cervical, thoracic and lumbar spine, chest and pelvis, the proximal portions of the femoral bones and in some cases frontal views of the chest. The BSC was classified as negative, doubtful or positive, using the following criteria:

Negative = Homogenous and symmetrical distribution of the radionuclide within the skeleton.

Doubtful = Slightly increased activity of radionuclide in one or multiple areas of markedly increased activity in one area only.

Positive = Markedly increased activity of radionuclide in at least two foci.

Bone marrow biopsy (BMB)

Bone marrow biopsies were taken from the posterior iliac crest with Gidlund's trephine (14) or an LM-trephine (10), under local anesthesia using Xylocaine^{R} (Astra) 20 mg/ml, 8 - 10 ml. Marrow specimens were fixed in 6 % formaldehyde and decalcified. After embedding them in paraffin, they were cut into 4 micron thick sections and stained with haematoxylin-eosin and with haematoxylin and van Gieson.

Patient distribution according to the clinical stage

BMB was performed in 103 patients in stage I - III at the time of initial diagnosis, in 312 patients in stage I - III during the followup 1/2 - 6 years after surgery and/or radiotherapy (Table 1), and in 207 patients in stage IV (Table 2). Of the 207 patients in stage IV, 96 had radiographically revealed osseous metastases only, 89 non-osseous metastases and 22 had mixed metastases (osseous and non-osseous).

None of the 96 patients with osseous metastases (Table 2) had clinically evident spread in other sites at the time of BMB and BSC.The distribution of metastases within the skeleton is shown in Table 3. Seventy-eight of these patients were also examined by BSC.

The group of 89 individuals with non-osseous metastases (Table 2) consists of 44 patients with microscopically verified cutaneous and/or sub-cutaneous metastases, 30 patients with radiographically revealed involvement of the lungs, 14 patients with metastases in the pleura and 1 patient with a metastasis in the pericardium. The pleural and pericardial metastases were cytologically verified. In 55 of these patients BSC was also carried out. Twenty-two patients had mixed metastases, Table 2. Ten of them had metastases in the lungs and 12 in the pleura. All of them had radiographically verified metastases in the ribs or spine, but not in the pelvis. In 17 of these patients BSC was also performed.

BMB was not performed in patients with liver, peritoneal or brain metastases.

The patients with clinical stage IV disease, positive BSC and positive BMB received combined chemotherapy ad modum COOPER, modified as follows: Oncovin 1 mg + Methotrexate 25 mg + 5-Fluorouracil 500 mg + Cyclophosphamide 500 mg i.v. once a week. Thirty-three patients also received Primobolan Depot^{R} (Methenolone-enanthate, Schering AB) 100 mg i.m. once a week. Some patients also received palliative radiotherapy.

Followup

The patients were followed up every other months with a physical examination, complete blood counts, control of serum electrolytes and liver function tests. Radiographic examination of the lungs and skeleton, bone and liver scintigraphy or other diagnostic procedures were performed when clinically indicated. All patients have been followed up for at least 40 months.

Statistics

The death risk was determined according to Mantel (12) using the time interval after surgical treatment as the time parameter of the risk function. Thus, the time after surgery was divided into nine intervals of one year's length. The cumulative survival was calculated in the following way: Within each interval the number of months of followup after BMB and BSC and the number of months followup were determined. Finally, the survival functions (S) were obtained from the hazard functions (h) by means of equation

$$S(t) = \exp - \int_0^t h(u)\, du \ .$$

RESULTS

Of the 415 patients with stage I - III tumors (Table 1) the BMB was positive in 30 negative in 385 patients. BSC was judged to be positive in 88 patients, negative in 73 patients and doubtful in 19 patients. The BMB was positive in 22 out of 88 patients with positive BSC, 3 in stage I, 11 in stage II and 8 in stage III. BMB confirmed 25 % of the bone metastases which were evident of BSC. Moreover, BMB revealed metastases in 6 out of 73 BSC negative patients (in 3 patients with stage II and in 3 with stage III tumors) Table 1.

Table 2 shows the result of BSC and BMB in 207 patients with stage IV tumors. Positive BMB in patients with positive BSC were obtained in 29 out of 73 patients with osseous metastases, 6 out of 24 patients with non-osseous metastases and 4 out of 14 patients with mixed metastases. Totally BMB confirm 35 % (39/111) of the bone metastases revealed by BSC. BMB revealed metastases in 5 out of 37 patients with negative BSC in stage IV carcinoma.

In all, BMB revealed metastases in 61 out of 199 (31 %) patients with stage I - IV carcinoma with positive BSC and in 11 out of 110 (10%) patients with negative BSC (Table 1 and 2),

Table 4 shows the death risk in 622 patients examined by BMB. The death risk of the patients with negative biopsies was compared with that of those with positive biopsies.

The death risk of the BMB-positive patients was greater than that of BMB-negative patients ($p < 0.0000001$, Table 4) but no difference in death risk existed between patients with positive and negative BSC ($p > 0.25$, Table 5).

The difference in survival between BMB-positive and BMB-negative patients is shown in Fig. 1. Fig. 2 shows the similarity of survival in BSC-positive and BSC-negative patients.

In order to elucidate the difference in prognostic value further, a special subgroup of 207 patients in whom BMB and BSC were performed within 5 months was studied. In 72 patients BSC was performed 1 month or less before BMB, in 50 patients BSC was performed within 1 month after BMB and in 85 ppatients the BSC was carried out within 4 months after BMB. The patients were studied with respect to survival after the latest of the two investigations. Once again a significant difference ($p < 0.0001$ Mantel's test, two-sided) was obtained for the BMB-negative patients compared with the BMB-positive patients (Table 6) and no significant difference was found when the BSC-negative patients were compared with the BSC-positive patients (Table 7). Twenty-three patients (12 %) died from intercurrent disease. Autopsy was performed in five of them and no evidence of cancer was found.

DISCUSSION

Early diagnosis of osseous metastases in breast cancer is of clinical importance. Besides the possibility of potential cure of solitary osseous metastases, effective palliative treatment of advanced disease by irradiation and chemotherapy can improve the patient's quality of life by preventing or decreasing invalidism and drug dependence due to chronic pain.

Generally, it is no doubt for the histopathological diagnosis of cancer cells in BMB. On the contrary, the clear-cut diagnosisin BSC is often much more difficult. A very important factor is the definition of a positive and doubtful diagnosis. It is the tendency not to accept single foci of increased activity, "hot-spots", as conclusive for the diagnosis of metastases (4, 7). Our results do not support this policy. Six out of 8 patients who had single foci of increased activity in the BSC showed microscopic metastases in BMB. Moreover, 11 out of 110 patients with negative BSC had metastases in the BMB from the posterior iliac crest. If benign lesions or inflammatory diseases can be excluded in patients with positive BSC, BMB should be taken from the areas with increased activity before suspicion of metastases is ruled out.Supplementary radiographic examination and, if necessary, tomography of an area with increased activity is also indicated. In this study 5 patients out of 118 (4 %) with positive radiograms had a negative BSC. This discrepancy was also observed by Lentle et al. 1975 (11), who found negative BSC in radiologically pure lytic lesions, probably due to decreased production of osteoid tissue.

Ingle et al. 1978 (9) studied 116 patients with primary and advanced breast cancer and correlated the results of BMB with those of radiographic examination and BSC. Positive BMB were found in 55 % of patients with positive radiograms, in 56 % of patients with positive BSC and in altogether 40 % of those with metastatic disease. This is a higher percentage than we found, 43 % (51/118 patients), 35 % (39/111 patients) and 28.5 % (59/207 patients), respectively, Table 3. Cohen et al. 1979 (3) found 26.3 % positive BMB in 38 patients with disseminated breast cancer.

Several factors may influence the results of BSC:

The diagnostic sensitivity of BSC varies with the isotope used. The best results were obtained with 99mTc stannous methylene diphosphonate, owing to faster urinary excretion, which lowers the background activity and increases the difference between bone and soft tissue activity.

The age of the patient also influences the diagnostic specificity and accuracy of BSC with respect to malignancy. Patients over 50 years and postmenopausal women have statistically more abnormal BSC than young and premenopausal individuals (2).

Moreover, a positive BSC reveals not only changes in the metabolism in the metastatic focus itself but also in the surrounding tissues. Inflammatory or degenerative diseases in the bone may therefore give a positive BSC simulating malignancy. This conclusion is supported by the study of Sklaroff and Charkes (15), who found carcinoma in 79 % of biopsies taken from BSC-positive skeletal foci, while 21 % remained microscopically negative, assuming that the biopsy technique was adequate.

Conflicting results concerning the prognostic value of BSC analysis have been reported. Galasko 1975 (6), Campbell et al. 1976 (2) and El-Dormieri et al. 1976 (5) emphasized the rationale for bone scanning in breast cancer and concluded that this technique provides an excellent prognostic index in the initial assessment of the disease. In contrast to these findings, there was no difference in survival between BSC-positive and BSC-negative patients in the studies of Blair 1975 (1) and Davies et al. 1977 (4).

Mendoza et al. 1969 (13) and Ingle et al. 1977 (8) studied the prognosis in patients with breast cancer with positive BMB. Ingle compared 15 BMB-positive and 28 BMB-negative individuals, Mendoza, using aspiration bone marrow biopsy, compared 41 patients with negative biopsies. The data presented in both studies show no correlation to the stage of the disease or the topography of the metastatic spread. In both studies the conclusion was drawn that BMB lacked prognostic value. This is not in agreement with our findings. The differency may be due to variation in the selection of patients and the relatively small numbers of patients in the previous studies.

In this study the lack of significance for BSC and the highly significant result for BMB may be interpreted as a difference in prognostic value. However, some objections may be raised against this interpretation. The size of the BMB group was twice as large as that of the BSC group. There might be some kind of selection of the BSC patients, who were a subgroup of the BMB group. BSC-doubtful patients were excluded. Furthermore in many individuals BSC was performed within 1 months prior to BMB.

These points of doubt in the statistical analysis could be ruled out by the evaluation of a subgroup of 207 patients in whom BMB and BSC were performed within 5 months. Even in this group BMB-positive had a significantly higher death risk than BMB-negative patients (Table 6). No significant difference in death risk between patients with positive and negative BSC could be detected (Table 7).

The prognostic significance of the BMB results may be due to several factors:

- The histopathological verification of metastatic osseous lesions in the biopsy is more specific than a positive BSC.
- The tissue volume examined by BMB from the iliac crest is minimal in comparison with the whole bone marrow mass. A positive tumor diagnosis by BMB probably presupposes extensive spread of cancer cells in the skeleton and other tissues, with a consequent high mortality.
- BSC, on the other hand, is a very sensitive but less specific method, revealing not only the real tumor mass in the skeleton, but also other bone marrow activity and changes in bone-blood flow.

With respect to our results BMB seems to be indicated as a diagnostic procedure for verification of metastases and for prediction of prognosis in all patients with positive BSC and in patients with negative BSC stage II - IV.

Table 1 CORRELATION BETWEEN BONE SCINTIGRAPHY (BSC)
AND BONE MARROW BIOPSY (BMB) IN PATIENTS WITH BREAST CANCER
STAGE I - III

n=415

STAGE		I n=60		II n=247		III n=108	
PATIENTS WITH		BMB pos.	BMB neg.	BMB pos.	BMB neg.	BMB pos.	BMB neg.
BSC	POSITIVE	3	11	11	32	8	23
	NEGATIVE	0	10	3	40	3	17
	DOUBTFUL	0	3	0	11	0	5
	NOT DONE	0	33	2	148	0	52

Table 2 CORRELATION BETWEEN BONE SCINTIGRAPHY (BSC) AND BONE MARROW BIOPSY (BMB) IN PATIENTS WITH BREAST CANCER STAGE IV

n=207

TYPE OF METASTASES		osseous n=96		non-osseous n=89		mixed n=22	
PATIENTS WITH		BMB pos.	BMB neg.	BMB pos.	BMB neg.	BMB pos.	BMB neg.
BSC	POSITIVE	29	44	6	18	4	10
	NEGATIVE	3	2	1	28	1	2
	DOUBTFUL	2	1	0	1	0	0
	NOT DONE	8	7	1	33	4	2

Table 3 LOCATION OF OSSEOUS METASTASES REVEALED BY RADIOGRAPHIC EXAMINATION

SKELETAL AREA	SOLITARY LESION	MULTIPLE LESIONS MAINLY IN
LUMBAR SPINE	23	27
THORACIC SPINE	13	4
RIBS	8	3
PELVIS	4	0
PROXIMAL FEMUR	5	1
CERVICAL SPINE	4	0
HUMERUS	4	0
TOTAL	61	35

TAB 4

DEATH RISK IN PATIENTS WITH BREAST CANCER
IN RELATION TO RESULTS OF BONE MARROW BIOPSY

YEARS AFTER SURGERY	BONE MARROW BIOPSY					
	NEGATIVE			POSITIVE		
	FOLLOW-UP MONTHS (a)	NO. OF DEATHS (b)	DEATH RISK ($b/a \times 10^4$)	FOLLOW-UP MONTHS (c)	NO. OF DEATHS (d)	DEATH RISK ($d/c \times 10^4$)
0 - 1	1652	6	36	138	5	362
1 - 2	3288	24	73	182	12	659
2 - 3	3715	25	67	246	8	325
3 - 4	3571	23	64	208	5	240
4 - 5	2370	18	76	200	5	250
5 - 6	1238	6	48	183	3	164
6 - 7	756	7	93	121	5	413
7 - 8	455	4	88	72	5	694
8 - 9	269	3	112	34	2	588
TOTAL		116			50	

$p < 0.0000001$, Mantel´s test, two-sided

TAB 5

DEATH RISK IN PATIENTS WITH BREAST CANCER
IN RELATION TO RESULTS OF BONE SCINTIGRAPHY

YEARS AFTER SURGERY	BONE SCINTIGRAPHY					
	NEGATIVE			POSITIVE		
	FOLLOW-UP MONTHS (a)	No. OF DEATHS (b)	DEATH RISK (b/a x 10^4)	FOLLOW-UP MONTHS (c)	No. OF DEATHS (d)	DEATH RISK (d/c x 10^4)
0 - 1	591	2	34	443	2	45
1 - 2	734	5	68	811	11	135
2 - 3	736	6	82	962	15	156
3 - 4	632	4	63	1009	9	89
4 - 5	421	3	71	851	9	106
5 - 6	191	1	52	607	5	82
6 - 7	86	3	349	415	6	145
7 - 8	29	3	1034	247	4	162
8 - 9	25	0	0	159	3	189
TOTAL		27			64	

p > 0.25, Mantel's test, two-sided

FIG. 1 PROBABILITY OF SURVIVAL IN PATIENTS WITH BREAST CANCER IN RELATION TO BONE MARROW BIOPSY DIAGNOSIS (BMB)

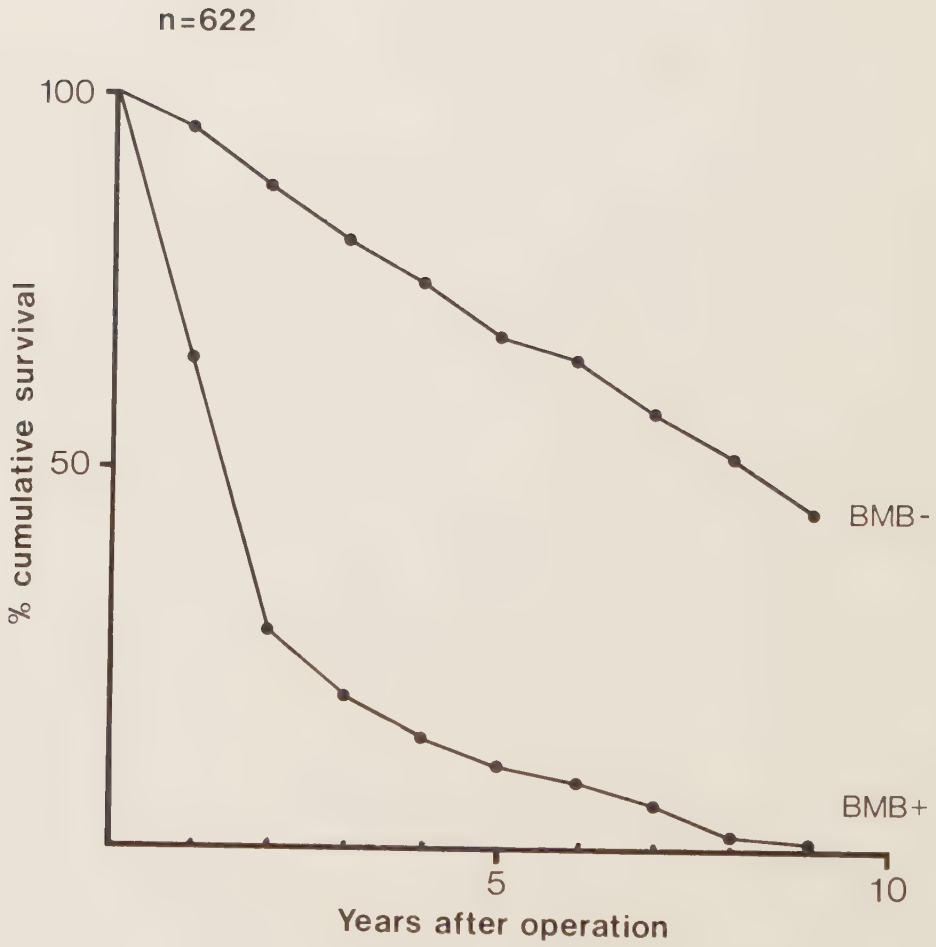
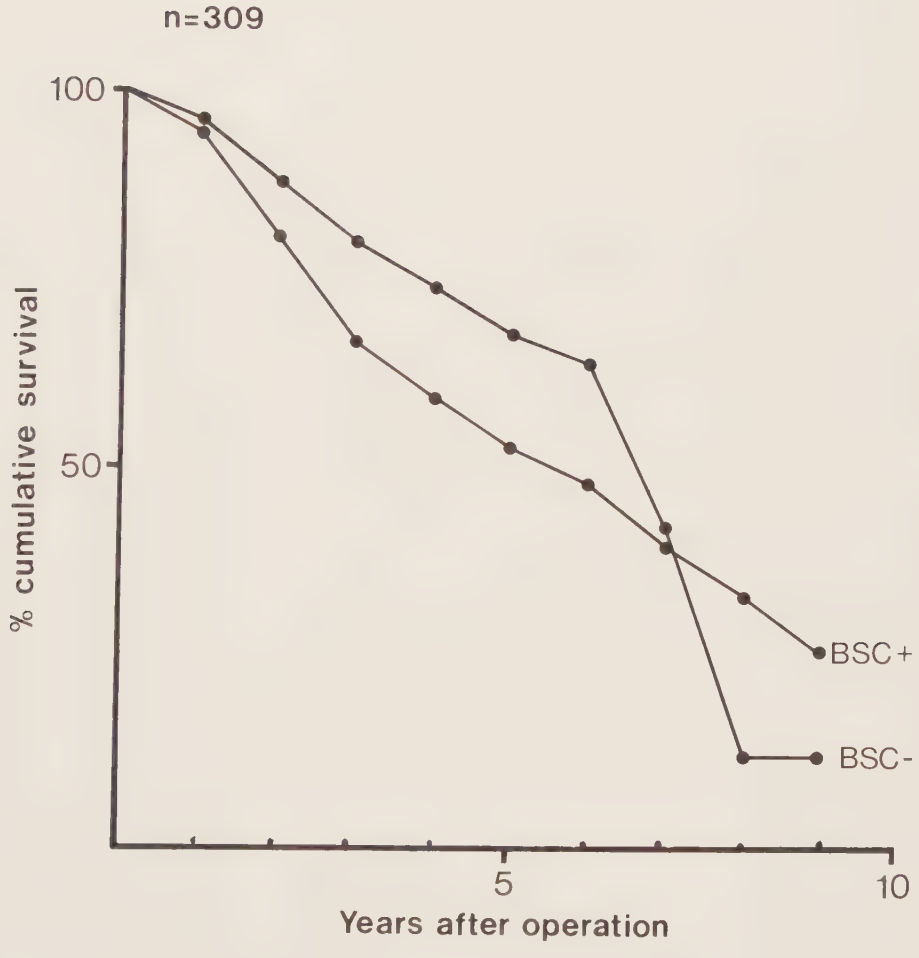


FIG. 2 PROBABILITY OF SURVIVAL IN PATIENTS WITH BREAST CANCER IN RELATION TO BONE SCINTIGRAPHY DIAGNOSIS (BSC)



TAB 6

DEATH RISK IN PATIENTS WITH BREAST CANCER IN RELATION TO RESULTS OF BONE MARROW BIOPSY

YEARS AFTER SURGERY	BONE MARROW BIOPSY					
	NEGATIVE			POSITIVE		
	FOLLOW-UP MONTHS (a)	No. OF DEATHS (b)	DEATH RISK ($b/a \times 10^4$)	FOLLOW-UP MONTHS (c)	No. OF DEATHS (d)	DEATH RISK ($d/c \times 10^4$)
0 - 1	421	1	24	104	3	288
1 - 2	745	7	94	132	8	606
2 - 3	819	10	122	183	5	273
3 - 4	817	6	73	120	1	83
4 - 5	517	4	77	107	4	374
5 - 6	267	1	37	79	0	0
6 - 7	186	2	108	52	1	192
7 - 8	114	2	175	39	1	256
8 - 9	102	1	98	34	2	588
TOTAL		34			25	

$p < 0.0001$, Mantel´s test, two-sided

TAB 7

DEATH RISK IN PATIENTS WITH BREAST CANCER IN RELATION TO RESULTS OF BONE SCINTIGRAPHY

YEARS AFTER SURGERY	BONE SCINTIGRAPHY					
	NEGATIVE			POSITIVE		
	FOLLOW-UP MONTHS (a)	No. OF DEATHS (b)	DEATH RISK ($b/a \times 10^4$)	FOLLOW-UP MONTHS (c)	No. OF DEATHS (d)	DEATH RISK ($d/c \times 10^4$)
0 - 1	277	2	72	248	2	81
1 - 2	422	5	118	455	10	220
2 - 3	420	3	71	582	12	206
3 - 4	367	2	54	570	5	88
4 - 5	193	1	52	431	7	162
5 - 6	82	0	0	264	1	38
6 - 7	53	1	189	185	2	108
7 - 8	32	2	625	121	1	83
8 - 9	27	0	0	109	3	275
TOTAL		16			43	

$p > 0.05$, Mantel´s test, two-sided

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PAPER V

HISTOPATHOLOGICAL PROGNOSTIC INDICATORS IN METASTATIC MAMMARY CARCINOMA IN THE BONE MARROW

By

B. Ridell, M.D., K. Landys, M.D. and A. Odén, Ph.D.

Departments of Pathology I and Radiotherapy, Sahlgrenska sjukhuset, University of Göteborg, Göteborg, Sweden.

Supported by grants from the Jubilee Clinic Foundation for Cancer Research, Göteborg, Sweden.

Correspondence to: Karl Landys, M.D.
Department of Radiotherapy, Jubileumskliniken
Sahlgrenska sjukhuset
S-413 45 Göteborg, Sweden

SUMMARY

The microscopical features of 78 iliac crest biopsies with metastatic mammary carcinoma were analysed semiquantitatively and the statistical correlation between different morphological traits and survival was established. A significant correlation was found between nuclear polymorphism and survival. No correlation could be found between other examined morphological traits and survival.

INTRODUCTION

The skeleton is a common site of metastases from mammary carcinoma and the prognostic importance of establishing spread to the bone marrow is well known. Bone marrow biopsy is not only a means of proving metastatic carcinoma in some instances, but also represents a means of obtaining tumor tissue for detailed morphological studies. This investigation was undertaken in order to investigate the prognostic value of semiquantitative examination of the tumor tissue in metastatic mammary carcinoma.

MATERIAL

Bone marrow biopsies were taken from 622 women with unilateral mammary carcinoma in clinical stages I - IV and with a varying duration of the tumor disease at the time of bone marrow biopsy.
Eighty-nine of these biopsies contained metastatic tumor and in 78 instances the amount and quality of the tumor tissue permitted a semiquantitative analysis. The biopsies were collected during a five-year period and the shortest individual followup for patients living at the time of termination of the study was 12 months. All individuals with morphologically established carcinoma in the bone marrow received cytostatic treatment as described previously (1), in some instances with addition of anabolic steroids (Primobolan Depot^{R}, Schering AG). All deceased individuals included in the study died from their tumor disease.

METHODS

Bone marrow biopsies were obtained and processed as described earlier (1,2).

The microscopical analysis comprised semiquantitative registration of the following morphological variables.

Variable 1 (V 1): The size of the metastasis was registered as "small" when the tumor tissue was focal and occupied less than 25 % of the bone marrow and "non-small" in other instances.

Variable 2 (V 2): The general growth pattern of the tumor tissue was registered as compact or disperse growth as described previously (1). In some biopsies both these types of growth occured and the growth pattern was then registered as mixed.

Variable 3 (V 3): The cell density of the tumor tissue was classed in four groups: less than 25 %, 25 - 50 %, 50 - 75 % and more than 75 % cells in the metastasis.

Variable 4 (V 4): The nuclear polymorphism was classed in four categories based on the degree of inequality in nuclear size, nuclear shape and chromatin density. The four categories of nuclear polymorphism were slight, moderate, marked and very marked (fig. 1 a - d).

Variable 5 (V 5): Structural differentiation was registered as present when obvious ducts, glands or papillary formations were found, otherwise as absent.

Variable 6 (V 6): The stroma reaction in- and outside the tumor tissue was registered as none, loose, collagen or mixed loose-collagen as described previously (1).

Variable 7 (V 7): The bone reaction was registered as none or slight, moderate or pronounced new bone formation. An accurate quantitation of the bone reaction could not be performed in the osteodestructive tumors and in the statistical analysis these were considered as missing data.
Variable 8 (V 8): Osteodestructive growth was registered as present when the tumor tissue affected the normal bone architecture and reduced the amount of trabecular bone, otherwise as absent.

The mitotic figures in the tumor were in most biopsies very sparse or absent. Partly for this reason and partly because of the difficulties in relating the mitotic number of cells in scirrhotic tumors, assessment of the number of mitoses was not done.

STATISTICAL METHODS

In order to investigate the correlation between some morphological variables describing the disease and survival, a special technique was developed. With this technique a survival score is defined. The score takes into account the fact that the length of the followup period varies individually and that some of the patients are alive and others are dead at the end of the followup. A non-parametric test based on the score was applied. In studies similar to the present one - in which a large portion of the patients died - the test may be expected to be more powerful than, for example, Mantel's test (3). Further details of the score and the test are given in the appendix.
Assuming that the hazard function of death h(t) is constant within one-month intervals, the maximum liklihood estimate of the survival function S(t) was obtained by means of the equation

$$S(t) = \exp - \int_0^t h(u)du$$

The function S(t) gives the probability of surviving t months after the taking of the biopsy.
Using the survival score, a multiple regression analysis was performed in order to find a set of morphological variables that can be used for predicting survival. Based on the fact that for each variable a combination of subgroups with a similar survival score would strengthen the regression analysis, and applying a rational approach, the following morphological variables were redefined for the regression analysis: V 3 (cell density) was divided into groups with cell density higher or lower than 75 %, V 6 (stroma reaction) was divided into groups with or without stroma reaction and V 7 with a very similar mean survival score in all subgroups, was omitted.

RESULTS

The number of individuals, the mean survival scores and statistical significances for each morphological group are shown in table 1. A statistically significant correlation was found between the degree of nuclear polymorphism and survival. all other correlations between single morphological variables and survival being non-significant. The survival functions for all individuals in the study and for the groups of subjects exhibiting a nuclear polymorphism of slight/moderate and marked/very marked degree respectively are shown in fig. 2. In the first group the survivors after 20, 40 and 60 months were 58, 37 and 12 % respectively and in the second group the survivors at the same time intervals were 30, 10 and 0 % respectively.
The results of the multiple regression analysis are given in table 2. It will be seen that the multiple correlation coefficient for the

most informative variable, nuclear polymorphism, was 0.30. Next to this variable, the two variables describing the mode of growth and tumor cellularity contained most information, raising the value of the multiple correlation coefficient to 0.37. Addition of further morphological variables gave only a slightly higher value for the multiple correlation coefficient, which was 0.39 when all analysed morphological variables were taken into account.

COMMENTS

In the metastatic lesions of mammary carcinoma both the tumor tissue and the surrounding fibrous and osseous tissue vary in quality, giving the metastatic lesions different micrscopical characteristics (1). In this investigation different structural traits have been analysed statistically with respect to their value for predicting survival. This analysis revealed one significant correlation, i.e. between the degree of nuclear polymorphism and survival. Several other morphological variables, such as mixed mode of growth, a high cellularity of the tumor tissue, absence of stroma reaction or osteodestructive growth, were associated with a shorter, but not significantly so, mean survival time.

However, semiquantitative analysis of the morphological traits with rather coarse grouping of the variables must necessarily be less accurate than continuous quantitative analysis and when such an analysis can be performed, other significant correlations may be revealed.

Several factors of importance for survival in mammary carcinoma are known (4). From morphological studies of the primary tumr, the histological type, the nuclear grade and the cellular host response are known to affect the prognosis (5 - 12). From the regression

analysis of this series of metastatic tumors, it seems that the basic properties of the tumor tissue are more important for predicting the prognosis than other microscopical phenomena, which may be the indirect effect of the tumor or considered a host response in the bone marrow and bone tissue.

The biopsy material studied came from a group of patients with widespread disease. Although the clinical staging had in some instances indicated a lower degree of spread, the bone marrow biopsy procedure led to restaging of the disease to stage IV. This means that in most instances the tumor disease was within or near the terminal phase. Prediction of survival from morphological studies of a metastatic lesion must necessarily be uncertain in this late phase of the disease since several other, more important, factors may contribute to the fatal outcome. Therefore, although a statistical correlation existed in the group studied, the nuclear polymorphism in bone marrow metastases must not be interpreted too rigidly in the individual patient, but merely regarded as having supplementary prognostic value in patients with terminal tumor disease.

APPENDIX

The following symbols will be used:

n = number of patients

X_i = length of time from biopsy to death of the i:th patient

Z_i = length of time from biopsy to the end of follow-up

S_i = variable indicating whether the i:th patient died or not during follow up.

The relation

$$Z_i \leq X_i$$

holds with equality if death occurs during follow-up. Define

$$S_i = \begin{cases} 0 \text{ if } Z_i < X_i \\ 1 \text{ if } Z_i = X_i \end{cases}$$

and the survival score v_i of the i:th patient by

$$v_i = \sum_{j=1}^{n} P(X_i > X_j \mid S_i, S_j, Z_i, Z_j)$$

where the conditional probabilities are determined from the estimated survival function. The conditional probabilities fullfill

$$P(X_i > X_j \mid S_i, S_j, Z_i, Z_j) = \begin{cases} 1 \text{ if } S_j = 1 \text{ and } Z_i > Z_j \\ 0 \text{ if } S_i = 1 \text{ and } Z_i < S_j \\ P(X_i > Z_j \mid X_i > Z_i) \text{ if } S_i = 0, S_j = 1, Z_i < Z_j \\ \frac{1}{2}P(X_i > Z_j \mid X_i > Z_i) \text{ if } S_i = S_j = 0, Z_i < Z_j \\ 1 - \frac{1}{2}P(X_j > Z_i \mid X_j > Z_j) \text{ if } S_i = S_j = 0, Z_i > Z \end{cases}$$

It can be proved that

$$\sum_{i=1}^{n} v_i = n(n-1)/2.$$

If all patients die, then $v_i + 1$ equals the rank of the survived time period of the i:th patient.

A non-parametric test of correlation between a variable U and survival is obtained by applying Pitman's test (13) to the observed sample $u_1, \ldots, u_n$ and the survival scores $v_1, \ldots, v_n$.

LEGENDS TO FIGURES

Fig. 1a - d. Microscopical appearance of tumors with a/ slight, b/ moderate, c/ marked and d/ very marked nuclear polymorphism.
Van Gieson x 380

Fig. 2. Survival functions for all individuals (A), the group with slight/moderate nuclear polymorphism (B) and the group with marked/very marked nuclear polymorphism (C).

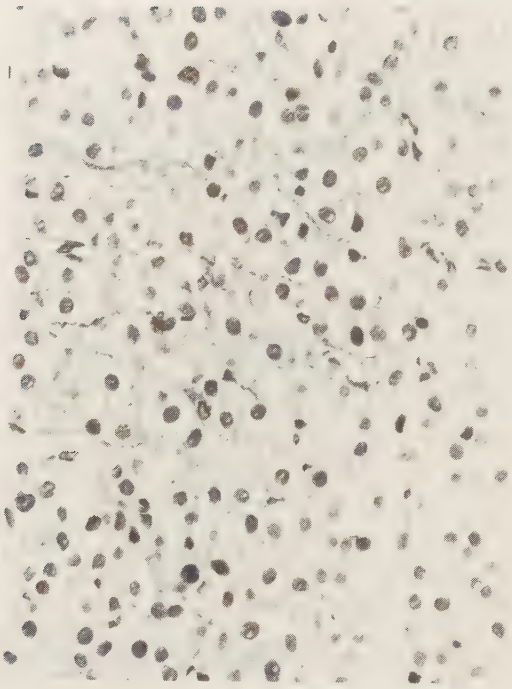

Fig 1 a

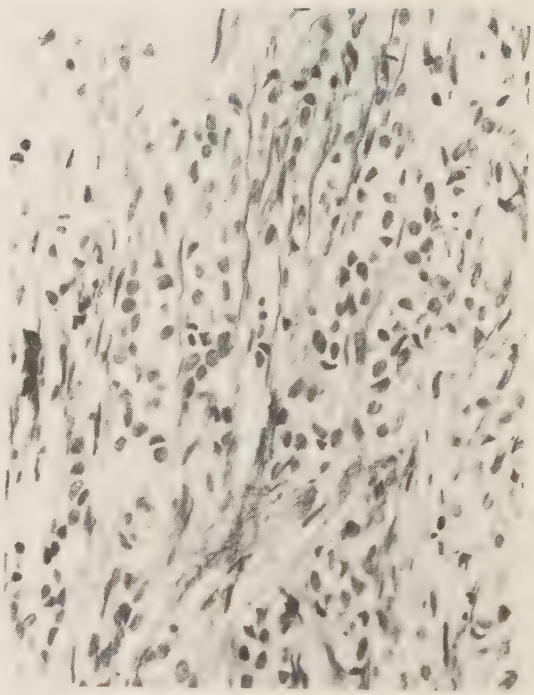

Fig 1 b

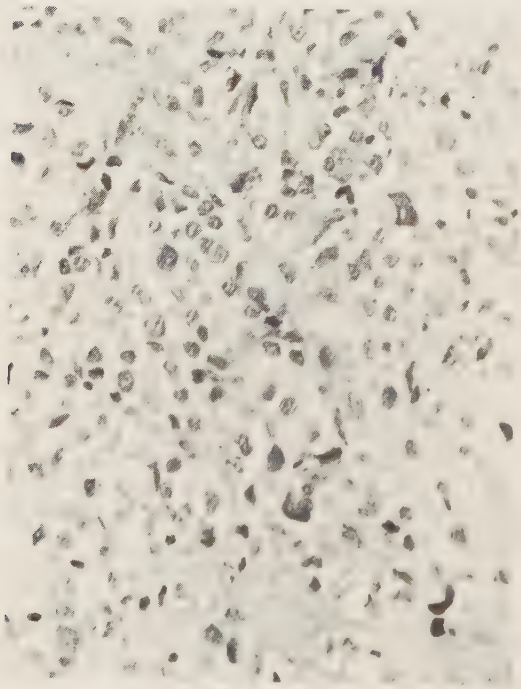

Fig 1 c

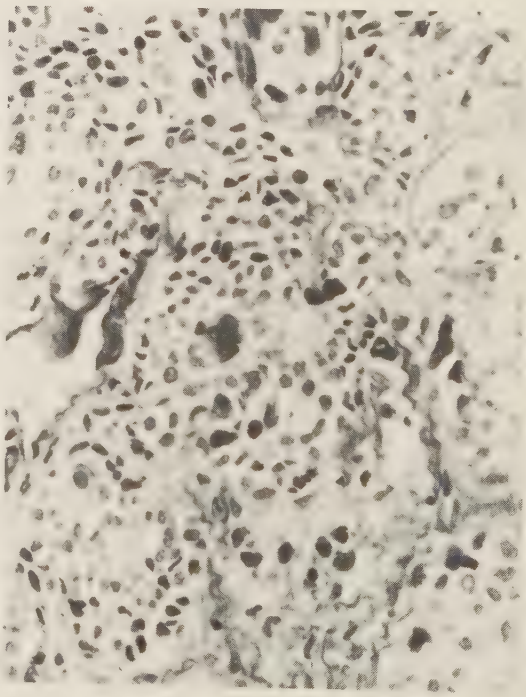

Fig 1 d

Fig 2

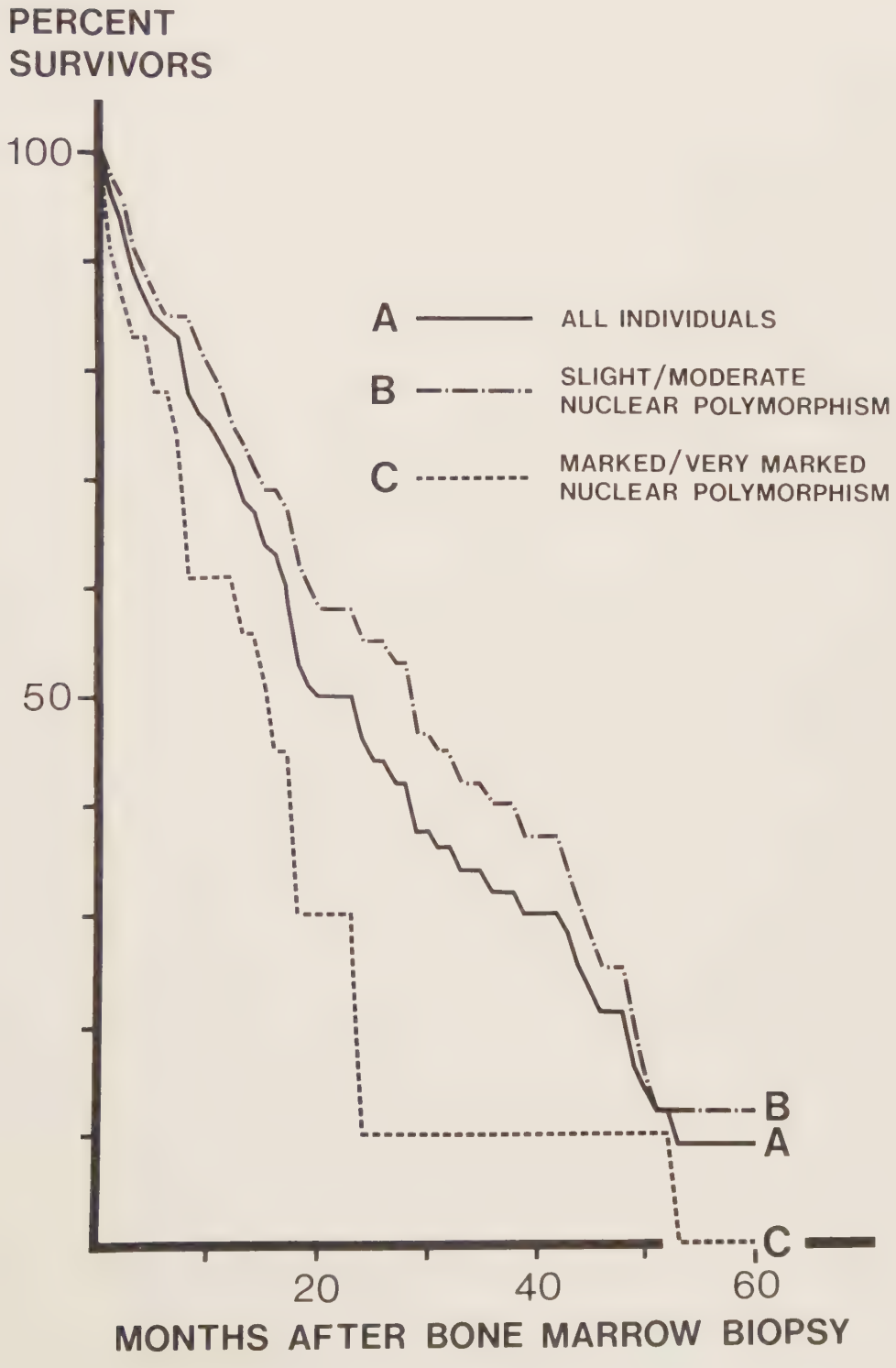
PERCENT
SURVIVORS
100
50
A
ALL INDIVIDUALS
B
SLIGHT/MODERATE
NUCLEAR POLYMORPHISM
C
MARKED/VERY MARKED
NUCLEAR POLYMORPHISM
B
A
C
20
40
60
MONTHS AFTER BONE MARROW BIOPSY

TABLE 1

Number of individuals, mean survival score and statistical significance for different morphological variables.

	Morphological variable		Number	Mean survival score	Statistical significance
V1	Size	Non-small	61	39,0	No
		Small	17	39,4	
V2	Mode of growth	Diffuse	14	43,6	No
		Compact	56	39,4	
		Mixed	8	29,2	
V3	Tumour cellularity	75 - 100%	20	31,8	No
		50 - 75%	12	41,5	
		25 - 50%	27	40,9	
		0 - 25%	19	42,9	
V4	Nuclear polymorphism	0	4	52,8	Yes ($2p < 0,01$)
		1	51	41,7	
		2	21	31,7	
		3	2	18,7	
V5	Structural differentiation	No	22	37,4	No
		Yes	56	43,6	
V6	Tumour stroma	None	10	32,7	No
		Loose	14	47,3	
		Mixed	14	40,4	
		Collagen	40	37,4	
V7	Bone reaction	None	19	38,7	No
		Slight	17	38,0	
		Moderate	17	43,4	
		Marked	13	42,9	
V8	Osteo-destruction	Yes	12	31,2	No
		No	66	40,6	

TABLE 2

Results of the multiple regression analysis

Number of variables	Regression function	Multiple correlation coefficient
One variable	S* = -11.05V4 + 54.14	0,30
Three variables	S = -6.03V2 - 8.34V3 - 9.89V4 + 65.41	0.37
Seven variables	S = 5.52V1 - 5.39V2 - 5.67V3 - 10.59V4 + 0.50V5 - 7.86V6 - 2.73V8 + 58.44	0.39

* S = survival score

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